SOILBORNE DISEASES RESEARCH CENTRE

Final Research Report

1st January, 1986 — 30th June, 1988

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DEPARTMENT OF BOTANY, UNIVERSITY OF KARACHI

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SUMMARY

During a survey of cultivated fields of Karachi and its suburbs, Thatta, Tandojam, Mirpurkhas, Sakrand, Nawabshah, Khairpur, Sukkur, Shikarpur, Jacobabad, Quetta, Lahore, Faisalabad and Rawalpindi-Islamabad areas, 52 new hosts of Rhizoctonia solani, 44 of Macrophomina phaseolina, 2 of Sclerotium rolfsii, 6 of Botryodiplodia theobromae and 3 of Melanospora sp., were recorded. Similarly, Drechslera australiensis on 44 hosts, D. halodes on 12 hosts and D. state of Cochliobolus spicifer on 6 hosts have been encountered for the first time in Pakistan. Damages due to root rot diseases produced a loss of upto 10-80% in different vegetable crops and fruit trees.

Experiments were carried out for the control of root rot and root knot infection caused by R. solani, M. phaseolina, Fusarium spp., and Meloidogyne spp., utilizing biological, chemical and cultural methods. Fungicides like Agrosan, Benomyl, Captan, Fernasan, and Vitavax @ 1,000 and 10,000 ppm inhibited growth of R. solani, M. phaseolina, Fusarium spp., S. rolfsii and B. theobromae in vitro. Agrosan and Fernasan when used as seed dressing showed 70% reduction in infection of R. solani on mung as compared to 50% reduction by Dithane and Vitavax and 40% by Kasumin. On okra 50% reduction of disease by Kasumin, 38% by Benomyl and 25% by Dithane was observed. Similarly, Fusarium infection on okra reduced by 38% in Benomyl, 16% in Captan, 18% in Vitavax and 11% in Kasumin treatments. Seed dressing with fungicides did not reduce infection by M. phaseolina. Agrosan, Benomyl, Captan and Vitavax drenched into soil were effective against R. solani even after 20 days of treatment while Dithane and Fernasan lost their efficacy after 10 days of treatment. A combined use of Benomyl and Captan was found effective against M. phaseolina, R. solani and Fusarium spp. Herbicides viz., Basfapon, Saturn and Stam significantly inhibited the in vitro growth of R. solani but @ 1,000 and 10,000 ppm were phytotoxic to okra when drenched into soil.

In vitro growth of M. phaseolina, R. solani and F. oxysporum was inhibited by several of the test organisms. Trichoderma hamatum and T. harzianum produced coiling around the hyphae of R. solani but not in M. phaseolina and Fusarium oxysporum. Seed dressing with antagonists using gum arbic as sticker reduced infection by M. phaseolina, R. solani and Fusarium sp., on bottle gourd, cotton, gram, mung, okra, snakemelon and sunflower. Microbial antagonists multiplied on rice grains when applied to soil produced better results than their use on wheat straw, rice straw, sorghum grains or as seed dressing. Paecilomyces lilacinus was found more effective than Furadan, Mucap, Agrosan and Vitavax in the control of root rot and root knot disease complex of mung, okra and tomato. A rice grain inoculum of P. lilacinus showed better results as compared to its use on wheat straw, rice straw, sorghum grain or as seed dressing. Persistence of the effect of P. lilacinus was greater than that of Furadan. P. lilacinus was more effective against root knot namatode Meloidogyne incognita than M. javanica.

A 15 days mulching treatment with transparent polyethylene sheets reduced the population of R. solani, M. phaseolina and Fusarium spp., to zero upto 15-20 cm depth. Plant growth was also better in mulched soil as compared to non-mulched soil. Organic fertilizers viz., cow dung, buffalo dung, goat dung, sewage sludge, Zarkhez, Ravi Pak and Chicken manure, significantly increased seed germination and reduced R. solani infection by upto 25-53% on okra, 50-67% on cotton, 14-100% on bottle gourd and 36-50% on mung. No significant reduction in M. phaseolina and Fusarium infection was observed. Combined use of microbial antagonists and organic fertilizers gave better results than their seperate use.

Diagnostic and advisory services for identification of root rot diseases and their control was provided. A training course on the "Diagnosis and Control of Soilborne Plant Diseases" was organized from 1-10 December, 1987, at the Department of Botany, University of Karachi in which 40 persons belonging to different research, extension and or education institutions of Pakistan participated.

INTRODUCTION

Plant disease produce serious losses to crop plants and adversely affect the agricultural economy of a country. Of the various diseases, the primary diseases threatening crop production are due to soilborne plant pathogens. The disease causing organisms like fungi, actinomycetes, bacteria and nematodes, which are ubiquitous and present in soil, infect roots of plants, limit nutrient uptake by plants and produce root rot disease complex resulting in death of the plants. Several of the soilborne disease causing fungi are difficult to eliminate since they produce resting structures like sclerotia, chlamydospores, etc., which are well adapted to survive for long periods under adverse environmental conditions. Since damage to plants by soilborne pathogens results from below ground infection, losses to crop plants from such diseases are underestimated and generaly go unnoticed (Baker & Cook, 1974). Examples of the pathogenic fungi involved in disease production are soilborne root infecting fungi like Macrophomina phaseolina, Rhizoctonia solani, Fusarium spp., and root knot nematode Meloidogyne spp., etc.,(Fig. 1). Of these, M. phaseolina is reported to produce charcoal rot of over 500 species of plants (Sinclair, 1982), R. solani exists as active mycelium in soil and attacks a tremendous range of plants (Parmeter, 1970). Similarly, Fusarium spp., (Booth, 1971) and Meloidogyne spp., (Sasser & Carter, 1985) are known to attack a wide range of plants. Results of our research investigations on soilborne root infecting fungi and their association with root knot nematode carried out from 1.1.1986 to 30.6.1988, are given in this report.

MATERIALS AND METHODS

1. Preparation of inocula

R. solani, M. phaseolina and Fusaium spp., were grown on wheat meal sand medium (5% w/w) for 4 weeks at 30°C. Inocula of R. solani and Fusarium were used as such whereas the sclerotia of M. phaseolina were separated after passing through 150 um (100 mesh) sieve. Clumps of sclerotia and mycelial fragments retained on the sieve were discarded and individual sclerotia free of mycelium that passed through the sieve were stored in sterilized glass vials at room temperature for subsequent studies. Viability of sclerotia was found 100% when plated on Potato Dextrose Agar (PDA).

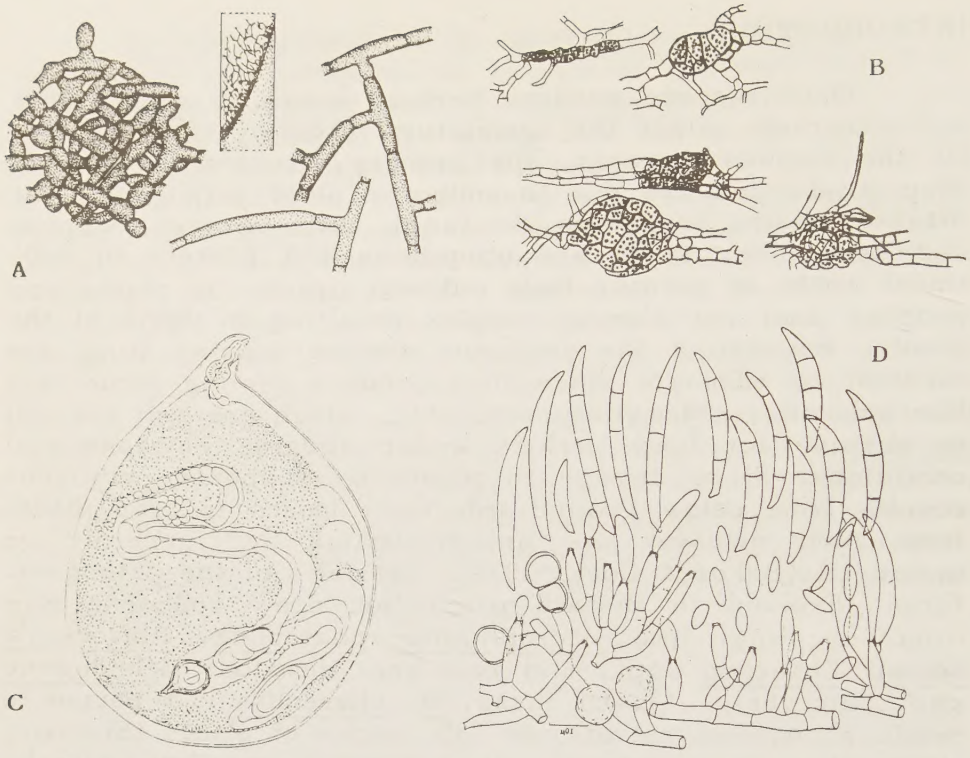

Fig. 1. Common soilborne root infecting pathogens

A= Rhizoctonia solani B= Macrophomina phaseolina C= Fusarium sp.
D= Meloidogyne sp.

2. Artificial infestation of soil

Soil used for artificial infestation was obtained from experimental plots of the Department of Botany, University of Karachi. The soil was sandy loam (sand,silt,clay; 70,19, 11%). pH ranged from 7.5-8.1 with moisture holding capacity (MHC) of 40% (Keen & Raczkowski, 1921), total nitrogen 0.077-0.099% (Mackenzie & Wallace, 1954), total organic matter 4.17-4.59%, initial total microbial population 3 x 10 fungal and 7 x 10 bacterial/actinomycete propagule /g of soil. Soil samples were air dried to 2-3% moisture and screened through 2 mm screen before use.

3. Isolation of fungi from soil

a. Soil dilution plate

Two g of soil sample was suspended in 18 ml sterilized distilled water which gave a dilution of 1:10. Using sterilized

pipette 2ml of soil suspension was mixed in 18 ml water and serial dilution of 1:100, 1:1,000 and 1:10,000 was prepared. Using a fresh sterile pipette 1 ml aliquot sample was poured in a 9 cm sterilized Petri dish and mixed into approximately 10 ml sterile PDA medium, pH 5.5. The dishes were incubated at 25°C. Fungi growing on dilution plates were counted and indentified. The number of colonies multiplied by the dilution factor gave total number of propagules /g of soil (Waksman & Fred, 1922).

b. Soil plate

Small soil sample (0.005-0.01 g) was dispersed in 1 ml of sterile distilled water in Petri dish and apparoximately 10 ml melted cooled PDA, pH 5.5, was poured and mixed. The soil particles were distributed throughout the medium by rotating the dish. The dishes were incubated at 25°C. Fungi growing in soil plate were identified (Warcup, 1950).

c. Baiting technique for isolation of R. solani

Sterilized millet grains were placed on top of the soil kept moist. The baits were removed, washed in water and transferred on agar medium for growth and identification of R. solani (Wilhelm, 1956). Percentage of colonized seeds was considered as population of R. solani in soil.

d. Wet sieving and dilution technique for isolation of M. phaseolina

Twenty g soil sample was wet sieved through 100 mesh (150 µm pore size) and 300 mesh (53 µm) screen. The residue obtained on 300 mesh screen was washed in running tap water for one minute and transferred into a beaker containing 0.5% $Ca(OCl)_2$ and made upto 100 ml to produce 1:5 dilution. The sclerotial suspension was put on a magnetic stirrer and 1 ml aliquot was evenly spread onto the surface of PDA plates containing penicillin and streptomycin each @ 60 mg/litre, Demosan 300 mg/litre and rose bengal 100 mg/litre. The plates were incubated at 30°C and in 5 days grayish to black colonies of M. phaseolina were identified (Sheikh & Ghaffar, 1975).

4. Isolation of fungi from roots

Roots were washed in running tap water, surface disinfected with 5% $Ca(OCl)_2$ and 1 cm pieces tranferred on

PDA containing penicillin and streptomycin. Dishes were incubated for 4 days at 28 °C to confirm infection and colonization of roots by soilborne root infecting fungi. Infection percentage and frequency of colonization was calculated as follows:

$$\text{Infection } \% = \frac{\text{Total number of plants infected by a pathogen}}{\text{Total number of plants}} \times 100$$

$$\text{Colonization } \% = \frac{\text{Total no. of root pieces colonized by a pathogen}}{\text{Total no. of root pieces of all plants}} \times 100$$

EXPERIMENTAL RESULTS

I. Survey and identification

A survey of the cultivated fields of Karachi and its suburbs, Thatta, Tandojam, Mirpurkhas, Sakrand, Nawabshah, Khairpur, Sukkur, Shikarpur, Jacobabad, Jhatpat, Quetta, Lahore, Faisalabad and Rawalpindi-Islamabad areas was carried out. Over 1000 plant samples belonging to 91 plant species were collected and microorganisms isolated from the roots of infected plants showing wilting or root rot disease complex. Fungi isolated were Rhizoctonia solani, Macrophomina phaseolina, Sclerotium oryzae, S. rolfsii, Fusarium spp., Botryodiplodia palmarum, B. theobromae, Colletotrichum capsici, Colletotrichum sp., Drechslera australiensis, D. halodes, D. hawaiiensis, D. state of Cochliobolus spicifer, Melanospora sp., and an unidentified basidiomycete (Table 1&2). In several cases a combination of root infecting fungi and root knot nematodes was found associated in the production of root rot disease symptoms and death of the plants.

R. solani was isolated from 64 plant species of which 52 host plants do not appear to have been recorded in Fungi of Pakistan (Mirza & Qureshi, 1978). Similarly, M. phaseolina was isolated from 67 different plant species of which 44 are new host records from Pakistan (Table 2). Species of Fusarium were isolated from 85 host plants. B. theobromae was recovered from 10 host plants which includes 6 new host records. B. palmarum was found to produce severe infection in coconut plantation in Karachi and its suburbs. Ginger was found to be a new host of Colletotrichum sp., not hitherto recorded. S. rolfsii isolated from barley and urid bean is reported for the first time from Pakistan. Similarly, D.

Table 1. SOILBORNE FUNGI LISTED UNDER THEIR HOST

1. Abelmoschus esculentus (L.) Moench
(Okra)
Curvularia tuberculata Jain
Drechslera australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
Macrophomina phaseolina (Tassi) Goid
Rhizoctonia solani Kuhn

2. Alhagi maurorum MediK.
C. tuberculata Jain
D. halodes (Drechsler) Subram. & Jain
Fusarium sp.

3. Allium cepa L.
(Onion)
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

4. A. sativum L.
(Garlic)
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

5. Amaranthus hybridus L.
(Smooth pigweed)
C. tuberculata Jain
Fusarium sp.
M. phaseolina (Tassi) Goid

6. A. virides L.
Fusarium sp.
M. phaseolina (Tassi) Goid

7. Anethum graveolens L.
(Dill)
D. halodes (Drechsler) Subram. & Jain
Fusarium sp.
M. phaseolina (Tassi) Goid
Nigrospora oryzae (Berk & Br.) Petch
R. solani Kuhn

8. Arachis hypogaea L.
(Groundnut)

Cephaliophora irregularis Thaxter
Chaetomium globosum Kunze ex Staud
Curvularia lunata (Wakker) Boedijn
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
D. state of Cochliobolus spicifer Nelson
Fusarium sp.
M. phaseolina (Tassi) Goid
N. oryzae (Berk & Br.) Petch
R. solani Kuhn
Unidentified basidiomycete

9. Artocarpus heterophyllus L.
(Jack fruit)

Botryodiplodia theobromae Pat.
Fusarium sp.
R. solani Kuhn

10. Avena sativa L.
(Oat)

D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
R. solani Kuhn

11. Beta vulgaris L.
(Sugarbeet)

Chaetomium sp.
C. tuberculata Jain
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
N. oryzae (Berk & Br.) Petch
R. solani Kuhn

12. Brassica oleracea L. var. capitata L.
(Cabbage)

C. globosum Kunze ex Staud
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

13. B. rapa L. (Pervirides group)
(Mustard)

D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

14. B. rapa L. (Rapifera group)
(Turnip)
C. lunata (Wakker) Boedijn
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

15. Capsicum annuum L.
(Pepper)
C. lunata (Wakker) Boedijn
C. tuberculata Jain
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

16. Carica papaya L.
(Papaya)
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

17. Carthamus oxycantha M.B.
M. phaseolina (Tassi) Goid

18. C. tinctorius L.
(Safflower)
M. phaseolina (Tassi) Goid

19. Centauria cyanus L.
Fusarium sp.
M. phaseolina (Tassi) Goid

20. Cicer arietinum L.
(Gram)
C. globosum Kunze ex Staud
C. tuberculata Jain
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
D. halodes (Drechsler) Subram. & Jain
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

21. Citrullus lanatus (Thunb.) Matsum. & Nakai
(Water melon)
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.

M. phaseolina (Tassi) Goid
R. solani Kuhn

22. Citrus limon (L.) Burm. f.
(Lemon)
D. halodes (Drechsler) Subram. & Jain
Fusarium sp.
Periconia sp.

23. Cocos nucifera L.
(Coconut)
B. palmarum (Cke) Pet & Syd.
Cephaliophora irregularis Thaxter
Chaetomium globosum Kunze ex Staud
Curvularia tuberculata Jain
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
D. sate of C. spicifer Nelson
Fusarium sp.
M. phaseolina (Tassi) Goid
N. oryzae (Berk & Br.) Petch
Periconia sp.
Phomopsis sp.
R. solani Kuhn
Unidentified basidiomycete

24. Corchorus olitorius L.
(Jute)
M. phaseolina (Tassi) Goid
Unidentified basidiomycete

25. Coriandrum sativum L.
(Coriander)
C. lunata (Wakker) Boedijn
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
D. halodes (Drechsler) Subram. & Jain
Fusarium sp.
M. phaseolina (Tassi) Goid
N. oryzae (Berk & Br.) Petch
R. solani Kuhn
Sclerotium sp.
Trichoderma sp.
Unidentified basidiomycete

26. Cucumis melo L.
(Muskmelon)
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.

M. phaseolina (Tassi) Goid
R. solani Kuhn

27. C. melo ssp. melo var. flexuousus (L.) Naudin
(Snakemelon)
C. globosum Kunze ex Staud
Corynascus sp.
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

28. C. sativus L.
(Cucumber)
B. theobromae Pat
C. tuberculata Jain
Fusarium sp.
M. phaseolina (Tassi) Goid

29. Cucurbita foetidissima H.B.K.
(Buffalo gourd)
D. australiensis (Bugni.) Subram.
& Jain ex M.B. Ellis
Fusarium sp.

30. C. pepo L.
(Pumpkins)
C. globosum Kunze ex Staud
Corynascus sp.
D. australiensis (Bugni.) Subram.
& Jain ex M.B. Ellis
Fusarium sp.
R. solani Kuhn

31. Cyamopsis tetragonoloba (L.) Taub.
(Guar)
C. globosum Kunze ex Staud
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

32. Cynodon dactylon (L.) Pers.
(Devil grass)
C. lunata (Wakker) Boedijn
D. australiensis (Bugni.) Subram.
& Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
N. oryzae (Berk & Br.) Petch
Unidentified basidiomycete

33. Cyperus bulbosus Vahl.
Fusarium sp.
M. phaseolina (Tassi) Goid

34. Dahlia variablis (Willd) Desf.
Fusarium sp.
M. phaseolina (Tassi) Goid

35. Daucus carota L. ssp. sativus (Haffm.) Arcang
(Carrot)
C. globosum Kunze ex Staud
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn
Stemphylium sp.

36. Dyssodia tenuiloba
Fusarium sp.
M. phaseolina (Tassi) Goid

37. Eruca vesicaria (L.) Cav. ssp. sativa (Mill.) Thell.
(Garden rocket)
C. globosum Kunze ex Staud
Fusarium sp.
N. oryzae (Berk & Br.) Petch

38. Euphorbia pulcherrima Willd. ex Kl.
(Poinsettia)
C. globosum Kunze ex Staud
Fusarium sp.
R. solani Kuhn

39. Ficus carica L.
(Fig)
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

40. Glycine max (L.) Merr.
(Soybean)
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

41. Gossypium arboreum L.
(Cotton)
C. globosum Kunze ex Staud
C. lunata (Wakker) Boedijn
C. tuberculata Jain
D. australiensis (Bugni.) Subram.
& Jain ex M.B. Ellis
D. state of C. spicifer Nelson
Fusarium sp.
M. phaseolina (Tassi) Goid
N. oryzae (Berk & Br.) Petch
R. solani Kuhn
Unidentified basidiomycete

42. **Helianthus annuus L.**
(Sunflower)

C. lunata (Wakker) Boedijn
C. tuberculata Jain
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
D. state of C. spicifer Nelson
D. halodes (Drechsler) Subram. & Jain
Fusarium sp.
M. phaseolina (Tassi) Goid
Melanospora sp.
N. oryzae (Berk & Br.) Petch
R. solani Kuhn
Sclerotium sp.

43. **Heliotropium curassavicum L.**
(Seaside heliotrope)

Chaetomium sp.
Fusarium sp.

44. **Hordeum vulgare L.**
(Barley)

Fusarium sp.
Sclerotium rolfsii Sacc.

45. **Lactuca sativa L.**
(Lettuce)

D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

46. **Lagenaria siceraria (Mol.) Standl.**
(Bottle gourd)

Chaetomium sp.
C. lunata (Wakker) Boedijn
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

47. **Lantana camara L.**
(Lantana)

Fusarium sp.
M. phaseolina (Tassi) Goid

48. **Luffa aegyptiaca Mill.**
(Sponge gourd)

C. lunata (Wakker) Boedijn
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis

Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

49. Lycopersicon esculentum Mill. (Tomato)
B. theobromae Pat.
Chaetomium sp.
C. lunata (Wakker) Boedijn
C. tuberculata Jain
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
D. halodes (Drechsler) Subram. & Jain
Fusarium sp.
M. phaseolina (Tassi) Goid
Melanospora sp.
N. oryzae (Berk & Br.) Petch
R. solani Kuhn
Unidentified basidiomycete

50. Mangifera indica L. (Mango)
B. theobromae Pat.
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn
Sphaeronema sp.
Unidentified basidiomycete

51. Manilkara zapota (L.) Van Royen (Chikoo)
B. theobromae pat.
Fusarium sp.
M. phaseolina (Tassi) Goid
Melanospora sp.

52. Medicago sativa L. (Lucern)
C. lunata (Wakker) Boedijn
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
N. oryzae (Berk & Br.) Petch
R. solani Kuhn
Unidentified basidiomycete

53. Melilotus alba Medik. (White sweetclover)
Fusarium sp.

54. **Mentha piperita** L.
(Peppermint)

Corynascus sp.
C. lunata (Wakker) Boedijn
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
Periconia sp.
R. solani Kuhn

55. **Mirabilis jalapa** L.
(Four O'Clock)

Colletotrichum sp.

56. **Momordica charantia** L.
(Bitter gourd)

D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

57. **Morus alba** L.
(Mulberry)

Fusarium sp.
R. solani Kuhn

58. **Musa paradisiaca** L.
(Banana)

D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

59. **Nicotiana tabacum** L.
(Tobacco)

Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn
Unidentified basidiomycete

60. **Oryza sativa** L.
(Rice)

B. theobromae Pat.
Chaetomium sp.
C. lunata (Wakker) Boedijn
C. tuberculata Jain
D. hawaiiensis (Bugni.) Subram. & Jain ex M.B. Ellis
D. state of C. spicifer Nelson
Fusarium sp.
M. phaseolina (Tassi) Goid
N. oryzae (Berk & Br.) Petch

R. solani Kuhn
Sclerotium oryzae Catt
Unidentified basidiomycete

61. **Pennisetum americanum (L.) Leeve**
(Millet)
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid

62. **Petunia violacea Lindl.**
(Petunia)
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

63. **Phaseolus lanatus L.**
(Lima bean)
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

64. **Piper betel L.**
(Betel)
B. theobromae Pat.
C. globosum Kunze ex Staud
Colletotrichum capsici (Syd.) Butl. & Bisby
C. lunata (Wakker) Boedijn
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
N. oryzae (Berk & Br.) Petch
R. solani Kuhn

65. **Pisum sativum L.**
(Pea)
Chaetomium sp.
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
D. halodes (Drechsler) Subram. & Jain
Fusarium sp.
R. solani Kuhn
Sclerotium sp.
Unidentified basidiomycete

66. **Portulaca oleracea L.**
(Purslane)
B. theobromae Pat.

D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
R. solani Kuhn

67. **Praecitrullus fistulosus (Stocks) Pangal**
(Gourd)
Chaetomium sp.
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
R. solani Kuhn
Periconia sp.

68. **Psidium guajava L.**
(Guava)
B. theobromae Pat.
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn
Trichodrma sp.
Unidentified basidiomycete

69. **Raphanus raphanistrum L.**
M. phaseolina (Tassi) Goid

70. **R. sativus L.**
(Radish)
Corynascus sp.
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

71. **Riccinus communis L.**
(Castor)
Fusarium sp.

72. **Saccharum officinarum L.**
(Sugarcane)
C. tuberculata Jain
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn
Trichoderma sp.

73. **Salsola baryosma (R. & S.) Dandy**
M. phaseolina (Tassi) Goid

74. Sesamum indicum L.
(Sesame)
C. lunata (Wakker) Boedijn
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn
Unidentified basidiomycete

75. Solanum melongena L.
(Egg plant)
C. lunata (Wakker) Boedijn
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

76. S. nigrum L.
(Black nightshade)
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
R. solani Kuhn

77. S. tuberosum L.
(Potato)
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

78. Sonchus oleraceus L.
(Common sowthistle)
Fusarium sp.
M. phaseolina (Tassi) Goid

79. Sorghum bicolor (L.) Moench
(Sorghum)
C. lunata (Wakker) Boedijn
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid

80. Spinacea oleracea L.
(Spinach)
C. globosum Kunze ex Staud
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
D. halodes (Drechsler) Subram. & Jain
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

81. **Tagetes erecta L.**
(Marigold)
Fusarium sp.
R. solani Kuhn

82. **Trachyspermum ammi** (L.) Spergue ex Turrill
Fusarium sp.

83. **Trifolium alexandrinum L.**
(Berseem)
Chaetomium sp.
C. lunata (Wakker) Boedijn
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
N. oryzae (Berk & Br.) Petch
R. solani Kuhn

84. **Trigonella foenum-graecum L.**
(Fenugreek)
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn

85. **Triticum aestivum L.**
(Wheat)
Chaetomium sp.
Corynascus sp.
C. lunata (Wakker) Boedijn
C. tuberculata Jain
D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
Fusarium sp.
M. phaseolina (Tassi) Goid
R. solani Kuhn
Sclerotium sp.
Trichoderma sp.
Unidentified basidiomycete

86. **Typha latifolia L.**
(Common cattail)
Fusarium sp.
N. oryzae (Berk & Br.) Petch
R. solani Kuhn
Trichoderma sp.
Unidentified ascomycete
Unidentified basidiomycete

87. **Vigna mungo** (L.) Hepper
(Urid bean)
C. lunata (Wakker) Boedijn
D. australiensis (Bugni.) Subram.

	& Jain ex M.B. Ellis
	Fusarium sp.
	M. phaseolina (Tassi) Goid
	Sclerotium rolfsii Sacc.
88. V. radiata (L.) Wilczek (Mung bean)	C. globosum Kunze ex Staud
	C. lunata (Wakker) Boedijn
	D. australiensis (Bugni.) Subram. & Jain ex M.B. Ellis
	D. halodes (Drechsler) Subram. & Jain
	Fusarium sp.
	M. phaseolina (Tassi) Goid
	R. solani Kuhn
89. Zea mays L. (Maize)	D. halodes (Drechsler) Subram. & Jain
	Fusarium sp.
	M. phaseolina (Tassi) Goid
	Memnoniella echinata (Rivolte) Galloway
	R. solani Kuhn
90. Zingiber officinale Roscoe (Ginger)	Arthrinium saccharicola Stev.
	Colletotrichum sp.
	Fusarium sp.
	N. oryzae (Berk & Br.) Petch
	R. solani Kuhn
91. Zinnia elegans Jacq. (Zinnia)	B. theobromae Pat.
	Fusarium sp.
	M. phaseolina (Tassi) Goid

Table 2. SOILBORNE FUNGI ASSOCIATED WITH VARIOUS CROPS

FUNGI	HOSTS	DISTRICT***
1. Botryodiplodia Sacc.		
B. palmarum (Cke) Pet & Syd. (1)		
	Cocos nucifera L.	1,2
B. theobromae Pat.		
	*Artocarpus heterophyllus Lam.	1
	*Cucumis sativus L.	10
	*Lycopersicon esculentum Mill.	1
	*Mangifera indica L.	1,4
	*Manilkara zapota (L.) van Royen	1
	*Oryza sativa L.	10
	Piper betel L.	1,2
	*Portulaca oleracea L.	1
	Psidium guajava L.	1,2
	Zinnia elegans Jacq.	1
2. Colletotrichum Corda		
C. capsici (Syd.) Butl. & Bisby (1)		
	Piper betel L.	1,2
Colletotrichum sp. (2)		
	Mirabilis jalapa L.	1
	*Zingiber officinale Roscoe	14
3. Drechslera Ito		
*D. austrliensis (Bugni.) Subram. & Jain ex M.B. Ellis (44)		
	*Abelmoschus esculentus (L.) Moench	1,3,5
	*Allium cepa L.	1,6
	*Arachis hypogea L.	1,15
	*Avena sativa L.	15
	*Beta vulgaris L.	1

*Brassica rapa L. (Perviridis group)	1,15
*Capsicum annuum L.	1,2,6
*Carica papaya L.	1
*Cicer arietinum L.	1,14,15
*Citrullus lanatus (Thunb.) Matsum & Nikai	1,10
*Cocos nucifera L.	1,2
*Coriandrum sativum L.	1,2,10,13
*Cucumis melo L.	1,3,10
*Cucurbita foetidissima H.B.K.	1
*C. pepo L.	10
*Cynodon dactylon (L.) Pers.	1,2,
*Gossypium arboreum L.	1,5,7
*Helianthus annuus L.	1,14
*Lactuca sativa L.	1,10,13
*Lagenaria siceraria (Mol.) Standl	1,6
*Luffa aegyptiaca Mill.	1
*Lycopersicon esculentum Mill.	1,2,6
*Mangifera indica L.	1,4
*Medicago sativa L.	1,13
*Mentha piperita L.	1,6
*Momordica charantia L.	1,6
*Musa paradisiaca L.	1,4
*Pennisetum americanum (L.) Leekel	1,3
*Petunia violacea Lindl.	1
*Piper betel L.	1
*Pisum sativum L.	1,14,15
*Portulaca oleracea L.	1
*Praecitrullus fistulosus (Stoces) Pangale	10
*Raphanus sativus L.	1,15
*Saccharum officinarum L.	3
*Sesamum indicum L.	1,15
*Solanum melongena L.	1
*S. nigrum L.	10
*Sorghum bicolor (L.) Moench	1,15
*Spinacia oleracea L.	1,13
*Triticum aestivum L.	6,7,10,15
*Vigna mungo (L.) Hepper	1,15
*V. radiata (L.) Wilczek	1

D. halodes (Drechsler) Subram. & Jain (12)

*Alhagi maurorum MediK.	2
*Anethum graveolens L.	1
*Cicer arietinum L.	14

*Citrus limon (L.) Burm. f.	1
*Coriandrum sativum L.	1,2
*Helianthus annuus L.	1,7
*Lycopersicon esculentum Mill.	1
*Medicago sativa L.	1,15
*Pisum sativum L.	14,15
*Spinacia oleracea L.	1,10
*Vigna radiata (L.) Wilczek	1
*Zea mays L.	1

D. hawaiiensis (Bugni) Subram. & Jain ex M.B. Ellis (1)

*Oryza sativa L.	10

***D. state of Cochliobolus spicifer Nelson (6)**

*Arachis hypogaea L.	1,15
*Cocos nucifera L.	1
*Gossypium arboreum L.	1,7
*Helianthus annuus L.	1,7,14
*Lycopersicon esculentum Mill.	1
*Oryza sativa L.	10,11

4. Fusarium Link. ex Fr. (85)

Abelmoschus esculentus (L.) Moench	1,3,5
Alhagi maurorum Medik.	2
Allium cepa L.	1,6
A. sativum L.	1,6
Amaranthus hybridus L.	10
A. viridis L.	1
Anethum graveolens L.	1
Arachis hypogaea L.	1
Artocarpus heterophyllus Lamb.	1
Avena sativa L.	15
Beta vulgaris L.	15
Brassica oleracea L. var. capitata L.	1,15
B. rapa L. (Pervirides group)	1,9,15
B. rapa L. (Rapifera group)	1,15
Capsicum annuum L.	1,6,9
Carica papaya L.	1
Centauria cyanus L.	1
Cicer arietinum L.	1
Citrullus lanatus (Thunb.) Matsum & Nakai	1,10

Citrus limon (L.) Burm. f.	1
Cocos nucifera L.	1,2
Coriandrum sativum L.	1,2,10,13
Cucumis melo L.	1,6,8,10
C. melo ssp. melo var. flexuousus (L.) Naudin	1,12
C. sativus L.	9,10
Cucurbita foetidissima H.B.K.	1
C. pepo L.	10
Cyamopsis tetragonoloba (L.) Taub.	1
Cynodon dactylon (L.) Pers	1,2
Cyperus bulbosus Vahl.	1
Dahlia variablis (Willd.) Derf.	1
Daucus carota L. ssp. sativus (Haffm.) Arcang	1
Dyssodia tenuiloba	2
Eruca vesicularia (L.) Cav. ssp. sativa (Mill.) Thell.	2
Euphorbia pulcherrima Willd. ex Kl.	1
Ficus carica L.	12
Glycine max (L.) Merr.	1,7
Gossypium arboreum L.	1,5,7
Helianthus annuus L.	1,7,14
Heliotropium curassavicum L.	10
Hordeum vulgare L.	1,2
Lactuca sativa L.	1,10
Lagenaria siceraria (Mol.) Standl	1,6
Lantana camara L.	1
Luffa aegyptiaca Mill.	1,2,6
Lycopersicon esculentum Mill.	1,2,6
Mangifera indica L.	1,4
Manilkara zapota (L.) vao Royen	1
Medicago sativa L.	1,2,5,10,13
Melilotus alba Medik.	2
Mentha piperita L.	1,6
Momordica charantia L.	1,6
Morus alba L.	1
Musa paradisiaca L.	1,4
Nicotiana tabacum L.	6
Oryza sativa L.	2,6,10
Pennisetum americanum (L.) Leeke	13
Petunia violacea Lindl.	1
Phaseolus lanatus L.	1
Piper betel L.	1,2
Pisum sativum L.	1,13,14,15
Portulaca oleracea L.	1,10
Praecitrullus fistulosus (Stock) Pangale	6,10

Psidium guajava L.	1,2
Raphanus sativus L.	1,15
Ricinus communis L.	1
Saccharum officinarum L.	2,3,6
Sesamum indicum L.	1,7,15
Solanum melongena L.	1,6
S. nigrum L.	10
S. tuberosum L.	1,13,14
Sonchus oleraceus L.	1
Sorghum bicolor (L.) Moench	1,11,13
Spinacia oleracea L.	1,10,13
Tegetes erecta L.	1
Trachyspermum ammi (L.) Spergue ex Turrill	10
Trifolium alexandrinum L.	1,2,13
Trigonella foenum-graecum L.	1,2,13
Triticum aestivum L.	1,2,6,8,10,15
Typha latifolia L.	2
Vigna mungo (L.) Hepper	1,2,15
V. radiata (L.) Wilczek	1
Zea mays L.	1,10,13
Zingiber officinale Roscoe	15
Zinnia elegans Jacq.	1

5. Macrophomina Petrak

M. phaseolina (Tassi) Goid (67)

Abelmoschus esculentus (L.) Moench	1,3,5
Allium cepa L.	1,4,6
A. sativum L.	1,6
**Amaranthus hybridus L.	1
**A. viridis L.	1
**Anethum graveolens L.	1
*Arachis hypogaea L.	1,15
*Beta vulgaris L.	1,13
*Brassica oleracea var. capitata L.	1,15
B. rapa L. (Pervirides group)	1,2,15
*B. rapa L. (Rapifera group)	1,15
*Capsicum annuum L.	1
Carica papaya L.	1
**Carthamus oxycantha M.B.	1
*C. tinctorius L.	3
*Centauria cyanus L.	1
*Cicer arietinum L.	1,14,15
*Citrullus lanatus (Thunb.) Matsum & Nakai	1

*Cocos nucifera L.	1
Corchorus olitorius L.	1
Coriandrum sativum L.	1,10,13
Cucumis melo L.	1,4,6,9,10
*C. melo ssp. melo var. flexuousus (L.) Naudin	1,10
*C. sativus L.	10,13
Cyamopsis tatragonoloba (L.) Taub.	1
**Cynodon dactylon (L.) Pers.	1,2
**Cyperus bulbosus Vahl.	1
*Dahlia variablis (Willd.) Derf.	1
*Daucus carota L. ssp. sativa (Haffm.) Arcang	1
**Dyssodia tenuiloba	2
*Ficus carica L.	12
Glycine max (L.) Merr.	1,3,17
Gossypium arboreum L.	1,3,5,7
Helianthus annuus L.	1
Lactuca sativa L.	1,13
Lagenaria siceraria (Mol.) Standl	1
*Lantana camara L.	1
*Luffa aegyptiaca Mill.	1
Lycopersicon esculentum Mill.	1,6
*Mangifera indica L.	1
*Manilkara zapota van Royen	1
Medicago sativa L.	1,5,6,10,13
Momordica charantia L.	1,6,9
Musa paradisiaca L.	1,3
*Nicotiana tabacum L.	6
**Oryza sativa L.	2
*Pennisetum americanum (L.) Leeke	13
*Petunia violacea Lindl	1
*Phaseolus lanatus L.	1
*Psidium guajava L.	1
**Raphanus raphanistrum L.	1
R. sativus L.	1,15
*Saccharum officinarum L.	2,3,6
**Salsola baryosma	1
Sesamum indicum L.	1,7,13
Solanum melongena L.	1,6
S. tuberosum L.	1,13
*Sonchus oleraceus L.	1
Sorghum bicolor (L.) Moench	1
*Spinacia oleracea L.	1,13
*Trifolium alexandrinum L.	1,2,4,15

*Trigonella foenum-graecum L.	1,2,13
**Triticum aestivum L.	1,3,9,15
*Vigna mungo (L.) Hepper	1,2,15
*V. radiata (L.) Wilczek	1,2
*Zea mays L.	1,10,13
*Zinnia elegans Jacq.	1

6. Rhizoctonia D.C.

R. solani Kuhn (64)

*Abelmoschus esculentus (L.) Moench	1,3,5
*Allium cepa L.	1,4,6
*A. sativum L.	1
*Anethum graveolens L.	1
*Arachis hypogaea L.	1,15
*Artocarpus heterophyllus Lam.	1
*Avena sativa L.	15
*Beta vulgaris L.	1
*Brassica oleracea L. var. capitata L.	1,15
*B. rapa L. (Perviridis group)	1,2,15
*B. rapa L. (Rapifera group)	1,15
Capsicum annuum L.	1,6
*Carica papaya L.	1
*Centauria cyanus L.	1
Cicer arietinum L.	1,14
*Citrullus lanatus (Thunb.) Matsum & Nakai	1,10
*Cocos nucifera L.	1,2
*Coriandrum sativum L.	1,13
Cucumis melo L.	1,2,6,10
*C. melo ssp. melo var. flexuousus (L.) Naudin	1,10
*Cucurbita pepo L.	1,10
*Cyamopsis tatragonoloba (L.) Taub.	1
*Daucus carota ssp. sativus (Haffm.) Arcang	1
*Euphorbia pulcherrima Willd. ex Kl.	1
*Ficus carica L.	12
*Glycine max (L.) Merr.	1,7
Gossypium arboreum L.	1,3,5,7
*Helianthus annuus L.	1,7
*Lactuca sativa L.	1,10
*Lagenaria siceraria (Mol.) Standl	1
*Luffa aegyptiaca Mill.	1
Lycopersicon esculentum Mill.	1,2,6

*Mangifera indica L.	1,4
*Medicago sativa L.	1,5,10,13
*Mentha piperita L.	1,6
Momordica charantia L.	1,6
*Morus alba L.	2
*Musa paradisiaca L.	1,4
Nicotiana tabacum L.	5
*Oryza sativa L.	8,9
*Petunia violacea Lindl	1
*Phaseolus lanatus L.	1
*Piper betel L.	1
*Pisum sativum L.	14
*Portulaca oleracea L.	1,10
*Praecitrullus fistulosus (Stocks) Pangale	6
*Psidium guajava L.	1
*Raphanus sativus L.	1
*Saccharum officinarum L.	3,4,6
Sesamum indicum L.	1,7,15
Solanum melongena L.	1,6
S. nigrum L.	10
S. tuberosum L.	1,13,14
*Sorghum bicolor (L.) Moench	1,11
*Spinacia oleracea L.	1,13
*Tagetes eracta L.	1
Trifolium alexandrinum L.	1,4,13,14
*Trigonella foenum-graecum L.	1,2,13
*Triticum aestivum L.	1,2,4,6,10,15
*Typha latifolia L.	2
*Vigna mungo (L.) Hepper	1,15
*V. radiata (L.) Wilczek	1
*Zea mays L.	1
*Zingiber officinale Roscoe	15

7. **Melanospora Corda**

Melanospora sp. (3)

*Helianthus annuus L.	1
*Lycopersicon esculentum Mill.	1
*Manilkara zapota van Royen	1

8. **Sclerotium Tode**

S. oryzae Catt. (1)

Oryza sativa L.	2

* S. rolfsii Sacc. (2)

*Hordeum vulgare L.	2
*Vigna mungo (L.) Hepper	2

Sclerotium sp. (4)

Coriandrum sativum L.	2
Helianthus annuus L.	7
Pisum sativum L.	14
Triticum aestivum L.	6

9. Unidentified basidiomycete (14)

Arachis hypogaea L.	15
Corchorus olitorius L.	1
Cocos nucifera L.	1
Cynodon dactylon (L.) Pers.	2
Gossypium arboreum L.	1,6
Lycopersicon esculentum Mill.	1
Mangifera indica L.	4
Medicago sativa L.	1
Nicotiana tabacum L.	6
Oryza sativa L.	10
Psidium guajava L.	1
Sesamum indicum l.	7
Triticum aestivum L.	2,6
Typha latifolia L.	2

*** 1. Karachi 2. Thatta 3. Tandojam 4. Mirpurkhas 5. Sakrand 6. Nawabshah 7. Khairpur 8. Sukkur 9. Shikarpur 10. Jacobabad 11. Jhatpat 12. Quetta 13. Lahore 14. Faisalabad 15. Rawalpindi-Islamabad.

** New host not hitherto recorded.

* New host record (pathogen or host) from Pakistan.

australiensis on 44 different hosts, D. halodes on 12 hosts, D. hawaiiensis on 1 host and D. state of C. spicifer on 6 hosts have been encountered. An unidentified Basidiomycetous fungus was found associated with roots of 14 host plants causing root rot diseases. Although fungi like Nigrospora oryzae, Chaetomium spp., and Curvularia spp., were isolated from several host plants but their role in the causation of root rot diseases needs study. Several new hosts of root knot nematode (Meloidogyne spp.) were also encountered which includes buffalo gourd, castor and prosopis as new hosts of M. javanica (Abid et al, 1988) and carrot, coriander, dill, lettuce (Sattar et al, 1987), rubber plant (Abid et al, 1988) and mustard (Shahzad & Ghaffar, 1987) as new hosts of M. incognita in Pakistan.

II. Extent of damage by soilborne diseases:

Root infecting fungi or a combination of fungi and root knot nematode was found to produce severe damages to plants. In the cultivated fields of Gadap and Malir, infection of coriander roots by M. phaseolina resulted in 10-20% mortality. Root rot of cotton crop showed upto 10% mortality at Sakrand. Upto 30-80% mortality of melon and water melon plants was observed in the cultivated fields of Malir, Landhi and Korangi where the disease was caused by a combination of soilborne root infecting fungi viz., M. phaseolina, R. solani and Fusarium spp. Similarly the yield of guar growing in Malir reduced by 30-40% as a result of infection of roots by root infecting fungi. A combination of root knot nematode (Meloidogyne spp.) and root infecting fungi viz., M. phaseolina, R. solani and Fusarium spp., produced more than 50% mortality of bitter gourd and sponge gourd plants in Malir. Yield of sugarbeet reduced by upto 20-30% as a result of root rot - root knot disease complex produced by an association of R. solani and M. incognita. Over 50% of the guava plants in an orchard in Malir showed severe die-back symptoms where an infection of root, shoot and fruits of guavas was found associated with B. theobromae. An association of B. palmarum and R. solani was found lethal for coconut plants in Gadap. Tomato crop in the cultivated fields of Malir, Landhi, Korangi and Gadap was invaribly affected by an association of root infecting fungi viz., M. phaseolina, R. solani, Fusarium spp., Drechslera spp., and B. theobromae, and root knot nematodes viz., M. incognita and M. javanica coupled with foliar infection by viruses. The tomato yield was greatly reduced and hardly there was a single unaffected plant.

III CONTROL EXPERIMENTS

a. Effect of fungicides

Use of fungicides for the control of fungal diseases is a common practice. There are several reports of reducing the disease caused by soilborne pathogens (Seoud et al, 1982; Suhag & Rana, 1984; Ilieseu et al, 1985) but in many cases especially in respect of sclerotial diseases, satisfactory control has not been achieved. The reasons that have been attributed are due to the longevity of fungal propagules in soil (Coley-Smith & Cook, 1971), the ability of many plant pathogens to atack their host over prolonged periods, the cost of chemicals involved and limited effectivenes of chemicals used to control root diseases (Rahe & Utkhade,1985). Experiments for the control of soilborne fungi were therefore caried out to study the effect of different fungicides in vitro and in vivo.

i. In vitro studies

Fungicides viz., Agrosan, Benomyl, Captan, Dithane, Fernasan and Vitavax @ 10, 100, 1,000 and 10,000 ppm were poured in 5 mm diameter holes made on Czapek Dox Agar and the test fungi viz., M. phaseolina, R. solani, Fusarium sp., B. theobromae and S. rolfsii were inoculated in the centre. The dishes were incubated at 28°C and inhibition in growth of fungi was recorded. Whereas Dithane showed no effect, Agrosan, Benomyl, Captan, Fernasan and Vitavax used @ 1,000 and 10,000 ppm were found most effective in suppressing growth of the fungi tested (Fig. 2).

ii. In vivo studies

1.Seed dressing

Seed treatment with fungicides has been the most economical method for preventing plant diseases in many crops. It can protect the seed from seed borne and soilborne pathogens during germination and emergence which may result in the establishment of a healthy seedling (Windels et al, 1985). Some of the fungicides selected from in vitro evaluation were therefore tested for seed dressing to study their effect in the control of soilborne root infecting fungi.

Seeds of mung bean (Vigna radiata (L.) Wilczek) and okra (Abelmoschus esculentus (L.) Moench) were treated with

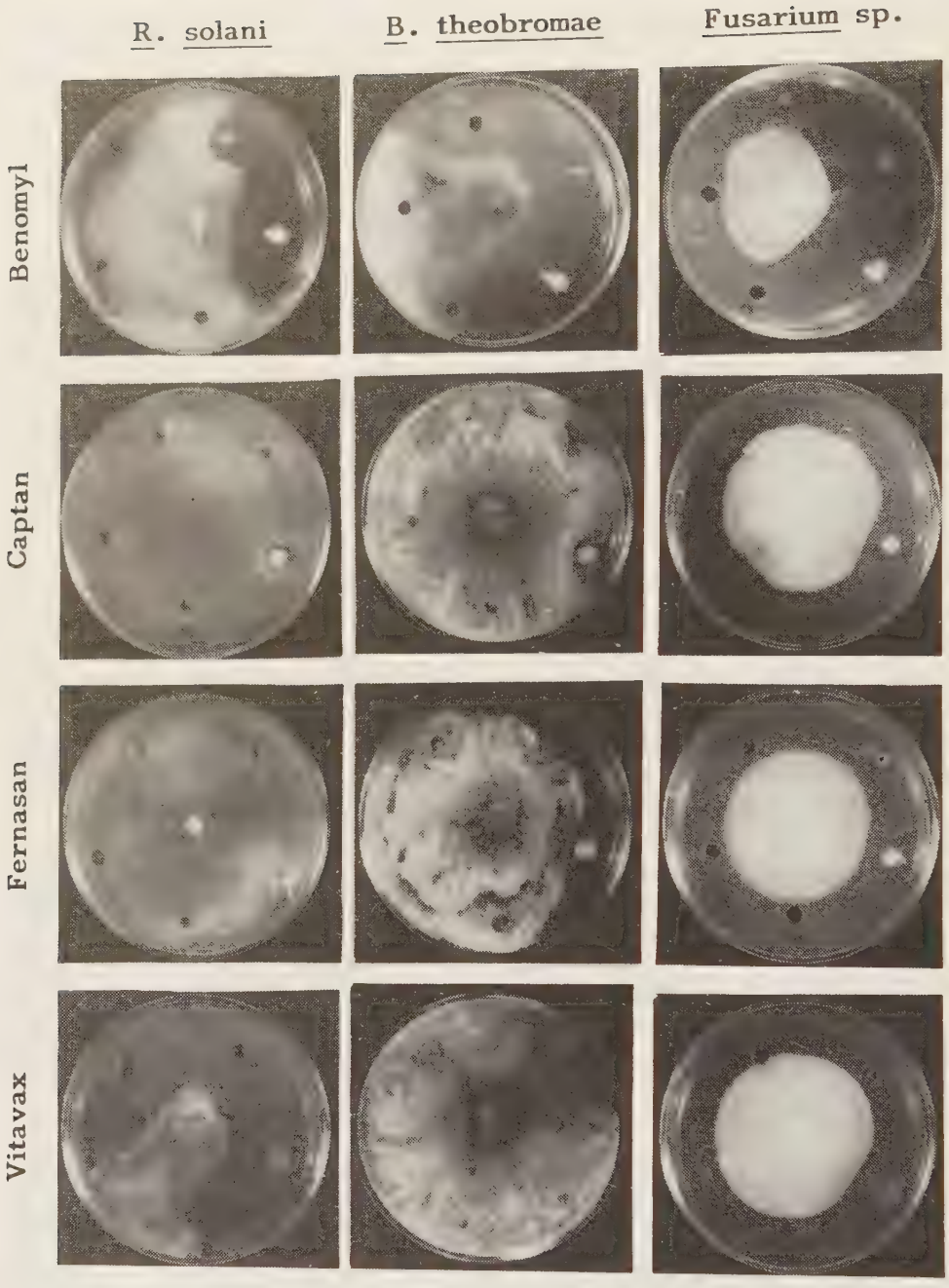

Fig. 2. Inhibition of Rhizoctonia solani, Botryodiplodia theobromae and Fusarium sp., with fungicides in vitro.

Holes in clockwise direction from the base contain fungicides @ 0, 10, 100, 1,000 and 10,000 ppm.

Agrosan, Dithane, Fernasan, Kasumin and Vitavax @ 4 g a.i./Kg seeds and planted in the field during May, 1986. After a period of 30 days the seedlings were uprooted to assess the infection and colonization of roots by M. phaseolina, R. solani and Fusarium spp.

Where mung was used Agrosan and Fernasan gave 70% reduction in infection of R. solani over control as compareed to 50% reduction in infection by Dithane and Vitavax and 40% by Kasumin (Fig. 3). On okra Fernasan and Kasumin

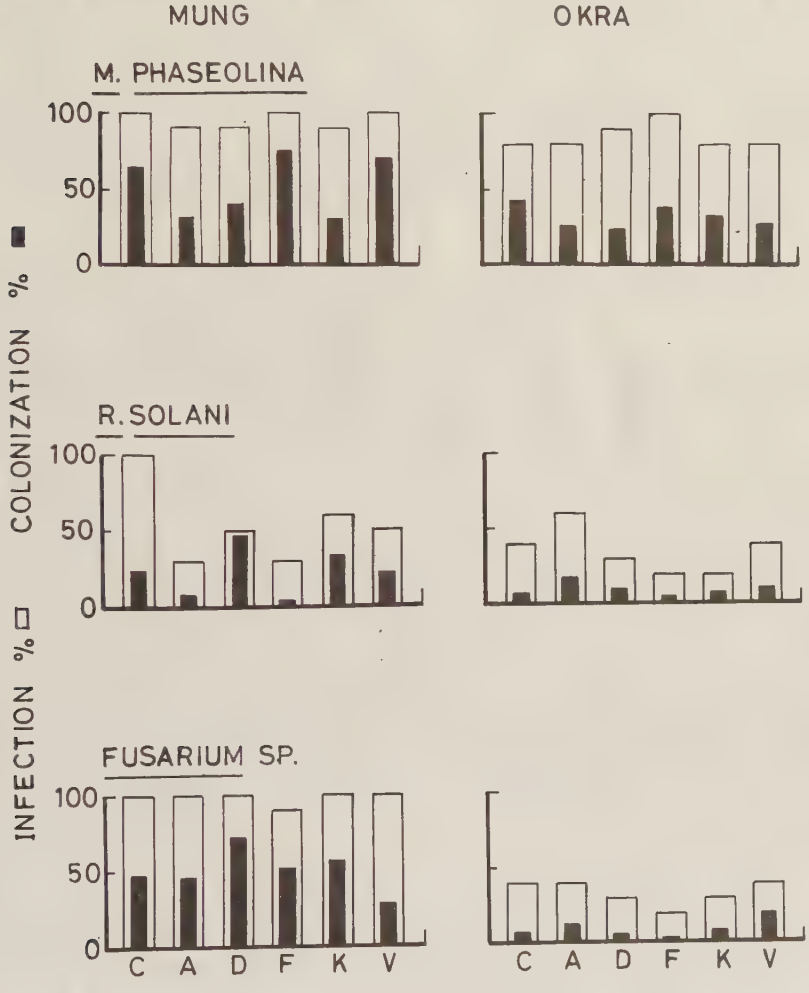

Fig. 3. Effect of seed dressing with fungicides on the infection and colonization of mung and okra by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp.

C= Control A= Agrosan D= Dithane F= Fernasan K= Kasumin V= Vitavax

reduced infection by 50% whereas Dithane resulted in 25% reduction in infection. Agrosan and Vitavax were unable to control R. solani infection on okra. Infection of mung bean roots by M. phaseolina reduced by upto 10% in Agrosan, Dithane and Kasumin treatments, the frequency of M. phaseolina colonization on roots respectively reduced by upto 50, 40 and 40% over the control. None of the fungicides used was able to reduce M. phaseolina infection on okra roots but the frequency of colonization reduced by upto 40, 47, 12, 26 and 37% respectively in Agrosan, Dithane, Fernasan, Kasumin and Vitavax treatments (Fig.3). Similarly infection on mung by Fusarium sp., reduced by 10% where Fernasan was used for seed treatment as compared to other fungicides which did not show any effect. Only Vitavax gave 40% reduction in the frequency of colonization. On okra reduction in infection by Fusarium sp., was 50% in Fernasan and 25% in Dithane and Kasumin. Seeds treated with Agrosan and Vitavax were unable to control infection of Fusarium sp., on okra (Fig. 3).

The effect of seed dressing with fungicides for the control of root rot infection was further examined during 1987 where seeds of okra (Abelmoschus esculentus (L.) Moench) were treated with Benomyl, Kasumin and Captan @ 4g/Kg seeds and sown at the experimental field of the Department of Botany, University of Karachi. After 30 days the plants were removed and 1 cm root pieces surface disinfected with Na(OCl) transferred on PDA and infection of roots by soilborne root infecting fungi recorded. Okra seeds treated with Benomyl and Kasumin, respectively, showed 45 and 25% reduction in R. solani infection whereas, seed treatment with Captan was ineffective against R. solani infection (Fig. 4). Infection by Fusarium spp., reduced by 38% (Benomyl), 16% (Captan) and 11% (Kasumin) as compared to control. Like 1986 trial, the fungicides used for seeed treatment were found ineffective against M. phaseolina and the infection increased in all the treatments as compared to control (Fig.4).

In another experiment seeds of okra were coated with Benomyl, Captan and Vitavax @ 4g/Kg seeds, while non-treated seeds served as control. Seeds were sown in the experimental plots at the Department of Botany, University of Karachi. After 30 days the plants were uprooted to assess the infection and colonization of roots by R. solani, M. phaseolina and Fusarium spp.

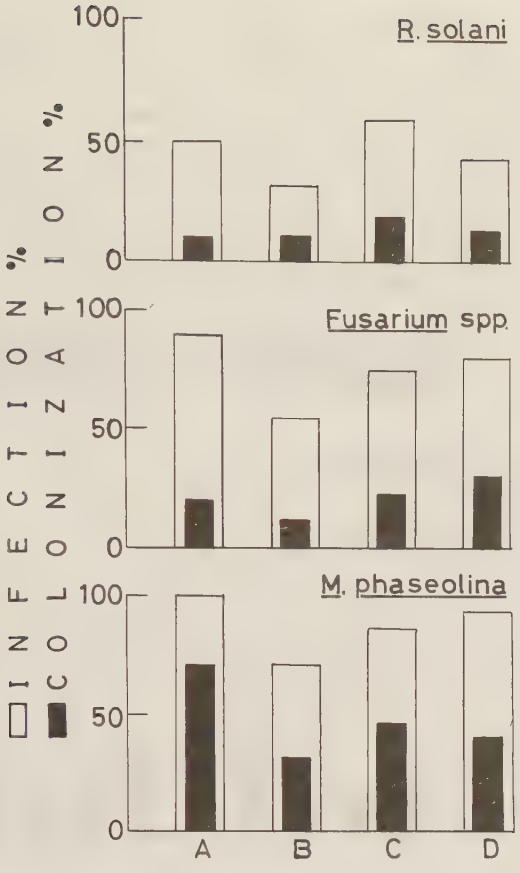

Fig. 4. Effect of seed treartment with fungicides on the infection of okra roots by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp.

A= Control B= Benomyl C= Captan D= Kasumin

More or less similar results were obtained since the reduction in R. solani infection was 41% by Benomyl and 25% by Vitavax whereas in Captan treated seeds the infection was 15% greater than the control. Similarly, reduction in Fusarium infection was 38% by Benomyl, 16% by Captan and 10% by Vitavax. No reduction in M. phaseolina infection was observed in any treatment. Observations taken after 60 days of sowing, however, showed an increase in R. solani infection whereas infection by Fusarium spp., reduced by 36% in Benomyl, 27% in Captan and 18% in Vitavax treatment. Similarly M. phaseolina infection reduced by upto 27, 14 and 17% respectively where Benomyl, Captan and Vitavax were used (Fig. 5).

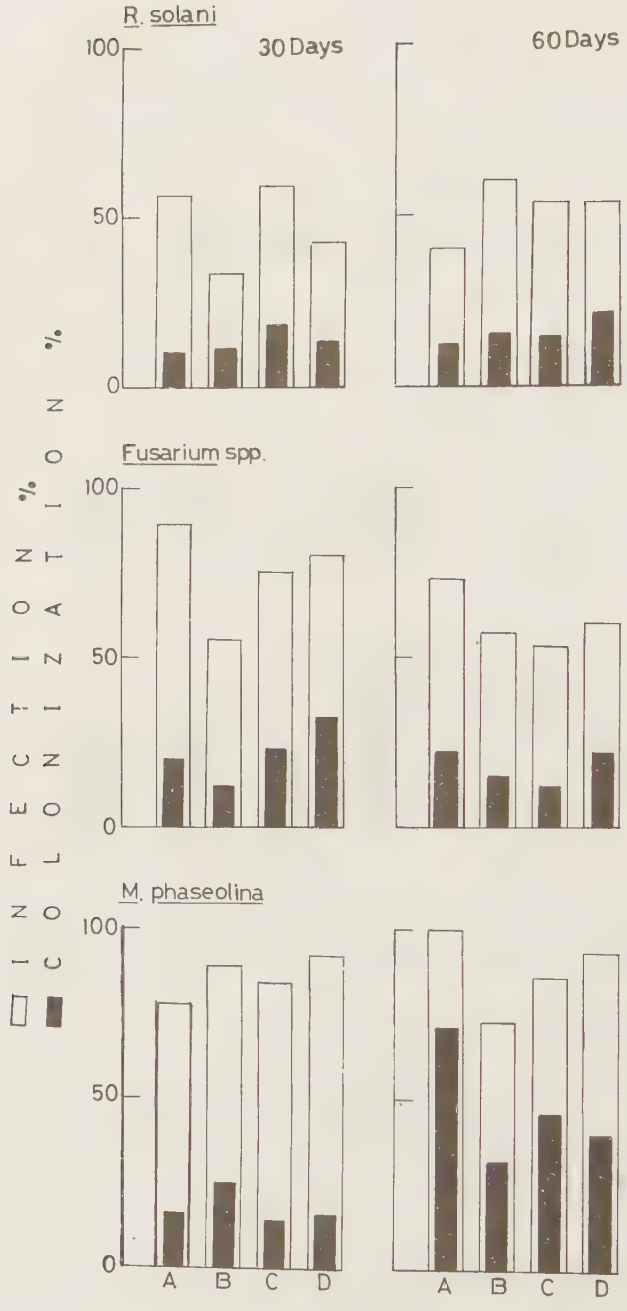

Fig. 5. Effect of seed treatment with fungicides on infection of okra roots by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp.

A= Control **B**= Benomyl **C**= Captan **D**= Vitavax

2. Soil drench

Since fungi are known to survive in the soil, experiments were carried out to study the effect of fungicides in eliminating soilborne infection. The fungicides in soil either inactivate the fungal propagules by adsorption and or degredation (Edgington et al, 1980). Experiments were therefore carried out where fungicides viz., Agrosan, Benomyl, Captan, Dithane, Fernasan and Vitavax were used as soil drench to study their effect on root infection by R. solani, M. phaseolina and Fusarium sp. The effective dose of fungicides and persistence of the effect of fungicides in soil was also examined.

Expt. i. R. solani was previously multiplied on soil wheat meal medium for 2 weeks. Soil artificially infested with R. solani @ 1% w/w was kept in 15 cm diameter pots and drenched with Agrosan, Dithane, Fernasan and Vitavax @ 10, 100 and 1,000 ppm in July, 1986. Non-treated soil served as control. Mung bean @ 10 seeds/pot was sown at 0-days and after 5, 10 and 20 days of treatment. Seedlings were uprooted after 30 days to assess R. solani infection.

Agrosan @ 100 and 1,000 ppm, Dithane and Vitavax at all concentrations and Fernasan @ 1,000 ppm showed 100% decrease in infection (Fig. 6). Agrosan and Vitavax used @ 100 and 1000 ppm retained their effectiveness even after 20 days of treatment while Dithane and Fernasan lost their efficacy after 10 days of treatment.

Expt. ii. In another experiment carried out during 1987, R. solani isolated from the roots of okra, was multiplied on soil wheat meal medium for two weeks. Sandy loam soil taken from the experimental field of the Department of Botany, University of Karachi was artificially infested with soil culture of R. solani @ 0.1% w/w, kept in 15 cm diameter plastic pots and drenched with Benomyl and Captan @ 10, 100 and 1,000 ppm. Non-treated soil served as control. Okra seeds @ 10 seeds/pot were sown at 0-day and after 5 and 20 days of treatment. Seedlings were uprooted after 30 days and infection of roots by R. solani was recorded.

Benomyl @ 100 ppm decreased R. solani infection by 89% in plants sown at 0-time and after 5 days of treatment and by 100% in plants sown after 20 days of treatment. Benomyl and Captan @ 10 ppm were found ineffective. Captan used @ 1,000 ppm reduced R. solani infection by 55 and 89% respectively when seeds were sown after 5 and 20 days of treatment (Fig. 7).

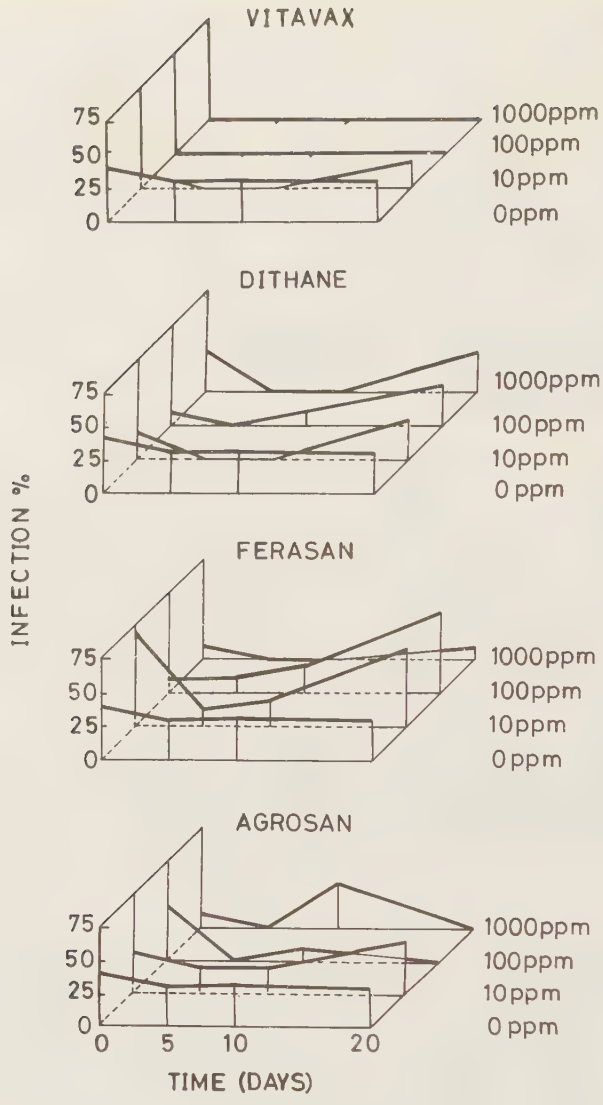

Fig. 6. Effect of soil treatment with fungicides on infection of mung bean roots by Rhizoctonia solani.

Expt. iii. In another experiment carried out during October, 1987, at the experimental plots of the Department of Botany, University if Karachi, soil was treated with Benomyl (@ 0.5 or 1 Kg/acre), Captan (@ 5 or 10 Kg/acre) or a mixture of Benomyl and Captan (@ 0.5 and 5 Kg/acre respectively). Seeds of sunflower (Helianthus annuus L.) were sown after 5 days of treatment and infection of roots by root infecting fungi assessed on 30 day old seedlings.

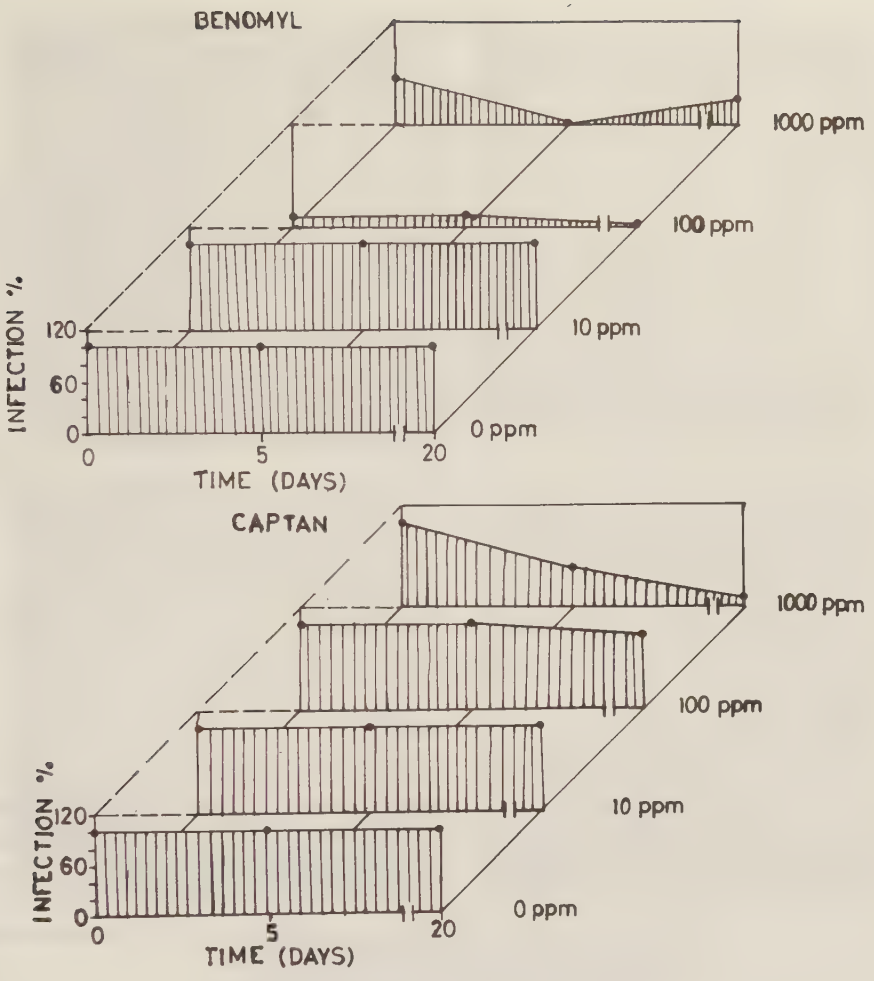

Fig. 7. Effect of soil application of fungicides on Rhizoctonia solani infection of okra roots.

Germination of seeds reduced where high doses of fungicides were used but there was no significant difference in germination in all treatments. Infection of root by R. solani reduced respectively by 34, 66 and 28% where Benomyl was used @ 0.5 or 1 Kg/acre or mixed with Captan. Captan used alone was found ineffective against R. solani. Root infection by M. phaseolina, respectively, reduced by 15, 22 and 40% in treatment where Captan was used @ 5 or 10 Kg/acre or mixed with Benomyl. Benomyl alone showed no reduction in M. phaseolina infection. Similarly Fusarium infection reduced by 22, 50 and 37% respectively, in soil treated with Benomyl (@ 1Kg/acre), Captan (@ 10 Kg/acre) or where Benomyl and Captan were used together. Benomyl and Captan at low doses were found ineffective against Fusarium sp., (Fig. 8).

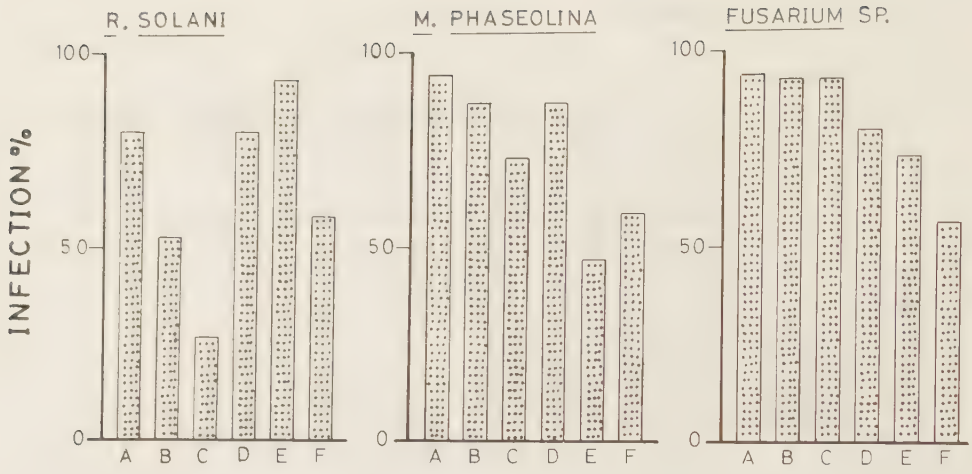

Fig. 8. Effect of soil treatment with fungicides on infection of sunflower roots by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp.

A= Control B= Benomyl @ 0.5 Kg/acre C= Benomyl @ 1 Kg/acre D= Captan @ 5 Kg/acre E= Captan @ 10 Kg/acre F= B + C

b. Effect of herbicides

Herbicides have been reported to affect the germination of fungal propagules and colonization of roots by soilborne root infecting fungi (Radke & Grau, 1986; Canaday et al, 1986; Rovira & Mc Donald, 1986). The effect of different herbicides on in vitro growth of R. solani and its infection on okra was therefore evaluated.

i. In vitro

Herbicides viz., Basfapon, Dymid, Machate, Saturn and Stam @ 10, 100, 1,000 and 10,000 ppm were poured in 5 cm diameter holes made on PDA, and R. solani inoculated in the centre. The dishes were incubated at 28°C and rate of growth of R. solani was recorded daily. Whereas Dymid and Machate showed no effect, Basfapon and Saturn used @ 1,000 and 10,000 ppm, and Stam @ 10,000 ppm significantly suppressed the growth of R. solani (Fig. 9).

ii. In vivo

Herbicides viz., Basfapon and Saturn @ 10, 100 and 1,000 ppm were drenched into the soil which was artificially

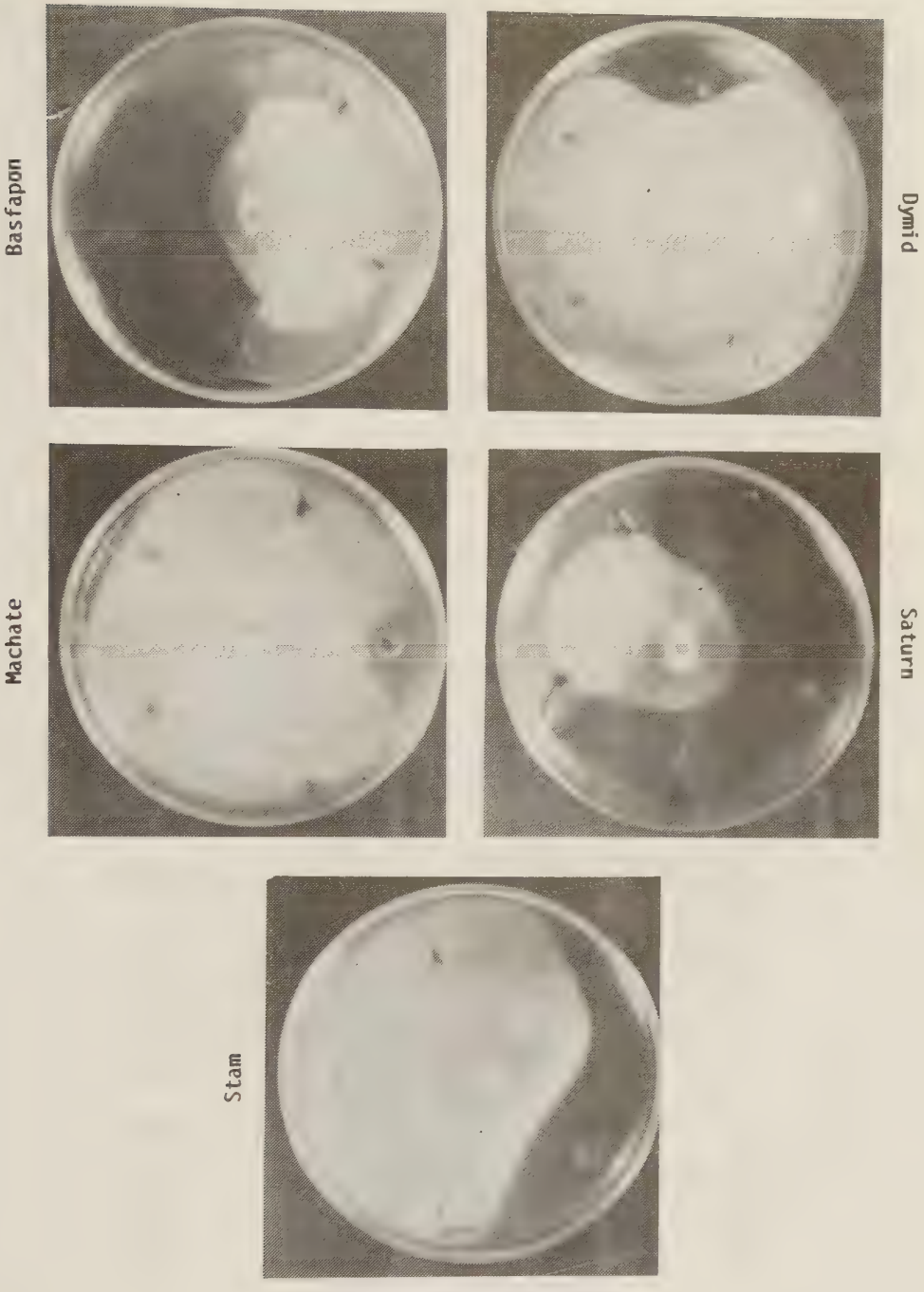

Fig. 9. Inhibition of Rhizoctonia solani with herbicides in vitro.

Holes in clockwise direction from the base contain herbicides @ 0, 10, 100, 1,000 and 10,000 ppm.

infested with R. solani @ 1% w/w. The experiment was carried out during March, 1986, where seeds of okra were sown at 0-time and after 5, 10 and 20 days of treatment. Infection of R. solani on roots of okra was assessed after 30 days growth of the seedlings. Basfapon when used @ 10 ppm showed no significant reduction in infection whereas it was phytotoxic when used @ 1,000 ppm. In plants sown at 0-time of soil treatment with Basfapon @ 100 ppm, infection of roots by R. solani reduced by 20% whereas , no reduction in infection was observed when seeds were sown after 20 days of treatment. Similarly, Saturn @ 10 and 100 ppm was ineffective and @ 1,000 ppm Saturn was phytotoxic since the plants died after 5 days of germination (Fig. 10).

c. Effect of microbial antagonists

Considering the cost of chemical pesticides and environmental hazards of the use of these chemicals, the

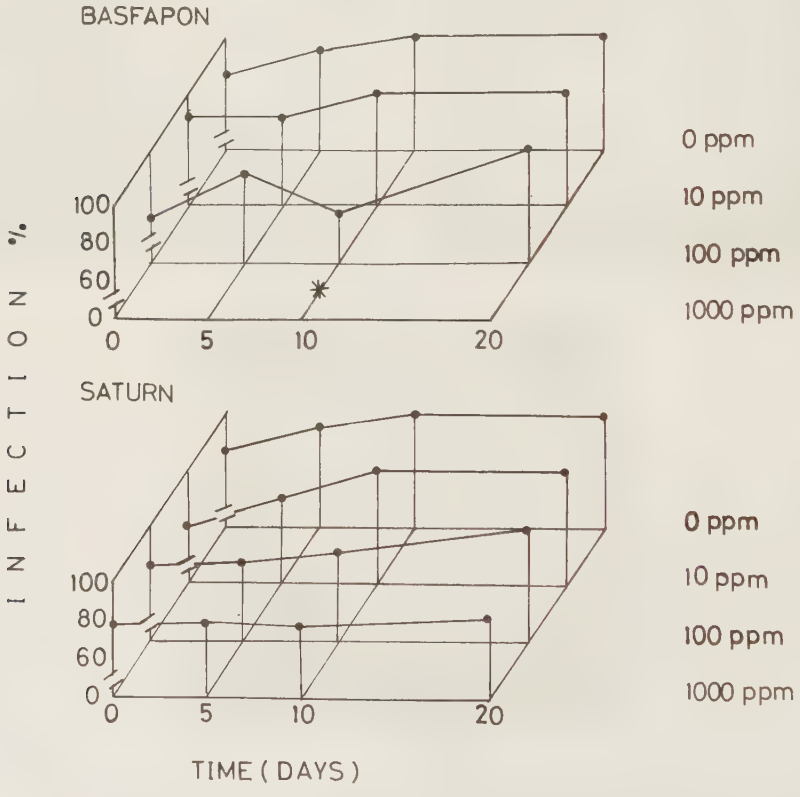

Fig. 10. Effect of herbicides on infection of okra roots by Rhizoctonia solani.

*= No germination.

use of microbial antagonists in the control of soilborne plant pathogens has received increasing attention throughout the world (Mulder, 1979). Several workers have used antagonistic microorganisms to control plant pathogens (Broadbent et al, 1971; Kommedahl & Mew, 1975; Windels & Kommedahl, 1978; Chet & Baker, 1981; Ghaffar, 1978, 1984). Of the antagonists Trichoderma spp., Gliocladium spp., and Laestisaria arvalis have been commonly used to control R. solani (Jager & Velvis, 1985). It may be mentioned that Gliocladium spp., and L. arvalis are not present in our soil (Mirza & Qureshi, 1978; Ghaffar & Kafi, 1968; Ghaffar et al, 1971; Ghaffar & Abbas, 1972). Experiments were therefore carried out where microbial antagonists native to our soil were isolated and these were used to study their effect on the in vitro growth of R. solani, M. phaseolina and Fusarium spp. Selective microbial antagonists were used to study their effect on the control of root infection by soilborne microorganisms.

1. Interaction of fungi with root infecting fungi

Using dilution plate (Waksman & Fred, 1922) and soil plate (Warcup, 1950) techniques a number of fungi, bacteria and actinomycetes were isolated from soil (Shahzad, 1984). In dual culture plate assays, Trichoderma hamatum and T. harzianum inhibited growth of R. solani which later over grew and produced a coiling around the hyphae of R. solani (Fig. 11). Arachniotus sp., Aspergillus candidus,

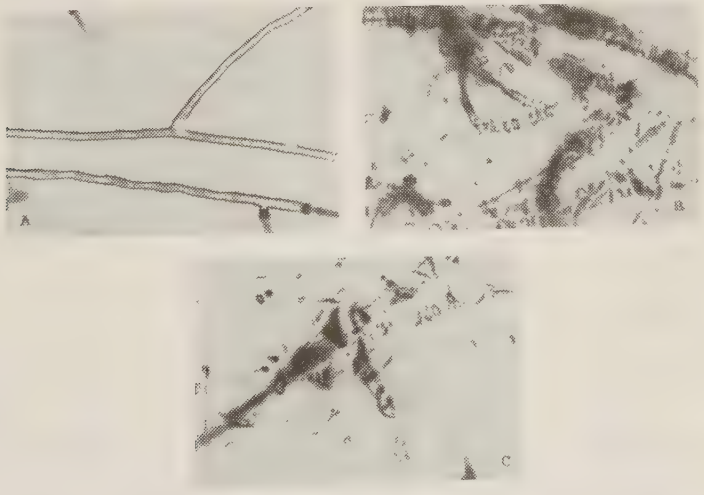

Fig. 11. Coiling of the hyphae of Rhizoctonia solani by Trichoderma spp.

A= Control B= Mycoparasitism by Trichoderma hamatum C= Mycoparasitism by T. harzianum.

Stachybotrys atra, Talaromyces flavus, Trichothecium roseum, Bacillus subtilis and Streptomyces sp., inhibited growth of R. solani producing zones of inhibition in vitro (Table 3). Similarly Arachniotus sp., A. candidus, A. fumigatus, A. niger, A. quadrilineatus, A. rugulosus, A. sulphureus, A. variecolor, Aureobasidium pullulans, Botryodiplodia theobromae, Chaetomium globosum, Cladosporium cladosporioides, Curvularia oryzae, C. pallesence, Drechslera halodes, D. hawiiensis, Fusarium chlamydosporium, F. culmorum, Memnoniella echinata, Nigrospora oryzae, Paecilomyces lilacinus, P. marquandii, Penicillium pupurogenum, Sclerotinia sclerotiorum, Scopulariopsis brevicaulis, Sordaria finicola, Stachybotrys atra, Stemphyllium lycopersici, Stysanus stemonitis, Talaromyces flavus and Trichothecium roseum produced a zone of inhibition against M. phaseolina (Table 4). Growth of Fusarium oxysporum was inhibited by

Table 3. INTERACTION OF R. SOLANI WITH FUNGI, ACTINOMYCETES AND BACTERIA IN VITRO

Test organisms	Days of incubation	Colony Diameter (mm)		Type of reaction*/ Zone of inhibition (mm)
		Pathogen	Test organisms	
FUNGI				
Arachniotus sp.	3	64	19	4
Aspergillus candidus	3	68	11	6
Chaetomium globosum	3	75	14	A
Cladosporium cladosporioides	3	76	6	
Memnoniella echinata	3	70	8	6
Penicillium purpurogenum	3	73	15	A
Stachybotrys atra	3	67	12	6
Stysanus stemonites	3	73	15	A
Talaromyces flavus	3	69	16	4
Trichoderma hamatum	3	53	44	B
T. harzianum	3	51	50	B
Trichothecium roseum	3	66	13	5
BACTERIA/ ACTINOMYCETES				
Bacillus subtilis	3	45	streaked	15
Streptomyces sp.	3	54	7	22

* A = Colonies meet each other. B = Test fungus over grows R. solani

Table 4. INTERACTION OF MACROPHOMINA PHASEOLINA WITH FUNGI IN VITRO

Test organisms	Days of incubation	Colony Diameter (mm)		Type of reaction*/ Zone of inhibition (mm)
		Pathogen	Test organisms	
Acremonium roseolum	5	60	30	B
Alternaria tenuis	4	58	32	B
Arachniotus sp.	4	55	30	5
Aspergillus candidus	4	54	22	10
A. flavus	3	54	38	B
A. fumigatus	5	50	35	5
A. glaucus	4	57	32	B
A. nidulans	4	61	29	B
A. niger	5	49	36	5
A. quadrilineatus	5	54	33	5
A. rugulosus	4	63	20	7
A. sulphureus	4	59	27	4
A. terreus	3	53	35	B
A. variecolor	5	56	31	4
Aureobasidium pullulans	4	63	16	5
Botryodiplodia theobromae	3	45	43	3
Chaetomium globosum	4	52	30	5
Cerciniella museana	3	45	48	C
Cldosporium cladosporioides	3	60	11	10
Cunninghamella echinulata	3	42	48	C
Curvularia lunata	4	53	37	B
C. oryzae	4	53	29	9
C. pallesence	4	55	31	4
C. tuberculata	3	47	43	B
C. vericulosa	3	48	42	B
Drechslera australiensis	4	53	39	B
D. halodes	3	52	32	6
D. hawaiiensis	4	47	36	8
D. rostrata	4	46	44	B
D. tetramera	4	60	30	B
Fusarium chlamydosporum	4	50	37	3
F. culmorum	5	61	22	8
F. lateritium var. buxi	4	55	35	B
F. semitictum	3	46	44	B
F. solani	3	47	43	B
Memnoniella echinata	5	59	10	21
Mucor corticosus	3	39	52	C

M. fragilis	3	41	49	C
Nigrospora oryzae	3	55	18	12
Paecilomyces lilacinus	4	55	15	20
P. marquandii	3	46	33	7
P. varioti	3	56	34	B
Penicillium purpurogenum	5	56	24	6
Rhizopus nigricans	3	39	60	C
Sclerotinia sclerotiorum	3	50	35	5
Sclerotium oryzae	3	50	50	C
Scopulariopsis brevicaulis	4	52	31	9
Sordaria finicola	4	61	20	9
Stachybotrys atra	4	61	13	14
Stemphylium lycopersici	4	48	35	8
Stysanus stemonitis	4	50	28	10
Syncephalastrum racemosum	3	45	50	C
Talaromyces flavus	4	71	14	4
Thamnidium avemalus	3	60	40	C
Trichoderma hamatum	3	37	58	A
T. harzianum	3	39	60	A
T. pseudokoningii	2	35	59	A
Trichorus spirilis	3	40	50	B
Trichothecium roseum	3	50	23	14

* A = Growth of M. phaseolina inhibited; test fungus over grew M. phaseolina colony.

B = Growth of test fungus inhibited; M. phaseolina over grew colony of the test fungus.

C = Colonies of M. phaseolina and test fungus intermingled and overlap each other.

Arachniotus sp., C. globosum, M. echinata, S. atra, T. flavus and T. roseum which produced a zone of inhibition against F. oxysporum (Table 5). It may be mentioned that R. solani has been found to be inhibited by Aspergillus clavatus (Wu, 1977), A. niger (Bora, 1977), Chaetomium cupreum (Yeh & Sinclair, 1980), Cylidrocarpon destructor, C. olivaceus, Epicoccum nigrum, F. culmorum and F. moniliforme (Chand & Logan, 1984), Penicillium sp., (Boosalis, 1956), T. roseum (Chand & Logan, 1984), Streptomyces sp., and B. subtilis (Elgoorani et al, 1976; Turchetti, 1979). Growth of M. phaseolina has been reported to be inhibited by Arachniotus sp., Aspergillus aculeatus, Cephalosporium humicola, F. moniliforme, Penicillium citrinum, P. variable, T. roseum (Dhingra & Khare, 1973), A. flavus (Jackson, 1965), A. niger (Vasudeva & Sikka, 1941), M. echinata (Abbas

Table 5. INTERACTION OF FUSARIUM SP. WITH FUNGI IN VITRO

Test organisms	Days of incubation	Colony Diameter (mm)		Type of reaction*/ Zone of inhibition (mm)
		Pathogen	Test organisms	
Arachniotus sp.	6	46	35	8
Aspergillus candidus	6	50	40	A
Chaetomium globosum	6	47	20	20
Cladosporium cladosporioides	8	65	15	A
Memnoniella echinata	6	51	21	12
Penicillium purpurogenum	8	56	34	A
Stachybotrys atra	6	52	10	14
Stysanus stemonitis	6	60	30	A
Talaromyces flavus	6	50	32	11
Trichoderma hamatum	4	32	58	A
T. harzianum	4	40	50	A
Trichothecium roseum	6	51	17	11

* A = Colonies meet each other.

& Ghaffar, 1975) and S. atra (Butt & Ghaffar, 1972). Similarly Fusarium spp., have been found to be inhibited by S. atra (Butt & Ghaffar, 1972), and B. subtilis (Cubeta et al, 1985).

2. Use of antagonists as seed dressing

The goal of biological seed treatment is to protect seed from seed borne and soilborne pathogens so as to enable the seed to germinate and become established as a healthy seedling (Chang & Kommedahl, 1968; Windels, 1981). These antagonists also presumably colonize the root surface and the presence of these in the rhizosphere provides protection against infection by root infecting pathogens (Chang & Kommedahl, 1968; Henis & Chet, 1975). Antagonists selected from in vitro screening were used to study their effect on the control of R. solani, M. phaseolina and Fusarium infection of gram, mung, mustard, snakemelon and sunflower roots.

Seeds of gram (Cicer arietinum L.), mung bean (Vigna radiata (L.) Wilczek), mustard (Brassica rapa L.), snakemelon (Cucumis melo ssp. melo var. flexuousus (L.) Naudin) and sunflower (Helianthus annuus L.) were coated with a spore suspension of 7 day old culture of antagonistic microorganisms

viz., A. candidus, T. harzianum, Streptomyces sp., and B. subtilis. Seeds were planted in naturally infested field during February, 1986. There were 100 seeds per treatment with 3 replicates of each. After a growth period of 30 days the seedlings were uprooted and 1 cm root pieces transferred on PDA to assess infection and colonization of roots by R. solani, M. phaseolina and Fusarium spp.

A. candidus significantly decreased R. solani root infection and frequency of colonization on all the plants tested, whereas T. harzianum reduced infection on gram (50%), mung (26%) and mustard (48%) only (Fig. 12). Streptomyces sp., and B. subtilis were effective on gram and mung but were unable to protect mustard, snakemelon and sunflower from R. solani infection. Similarly T. harzianum reduced infection of roots by M. phaseolina on mung (67%), mustard (31%) and gram (63%) whereas Strptomyces sp.,reduced M. phaseolina infection on mung (15%), mustard (15%), gram (16%) and snakemelon (100%). B. subtilis reduced root infection by M. phaseolina on mung (56%) and mustard (46%) whereas A. candidus was found effective only on mustard, reducing M. phaseolina infection by 76% (Fig. 12). Surprisingly none of the antagonist was able to protect sunflower roots from M. phaseolina infection. Reduction in infection of Fusarium spp., by A. candidus was 33% on mung, 31% on mustard, 50% on snakemelon and 24% on sunflower, whereas T. harzianum resulted in 18 and 50% reduction in infection on gram and snakemelon (Fig. 12). Streptomyces sp., reduced infection of Fusarium sp., by 30% on gram and 38% on snakemelon but it was unable to reduce infection on mung whereas frequency of colonization was reduced by 38%. B. subtilis was able to reduce the infection of Fusarium on gram, mustard and snakemelon roots by 27, 24 and 50%, respectively.

In another experiment seeds of mung bean (Vigna radiata (L.) Wilczek) were treated with microbial antagonists viz., Arachniotus sp., Aspergillus candidus, Paecilomyces lilacinus, Talaromyces flavus, Trichoderma hamatum, T. harzianum, Streptomyces sp.,Bacillus subtilis, Rhizobium leguminosarum and R. meliloti and planted in the field during May, 1986. Non-treated seeds served as control. Gum arabic (1%) was used as sticker for seed dressing by the test organisms.

All of the antagonists used gave substantial reduction in infection over control (Fig. 13). Reduction in infection by R. solani was 100% by P. lilacinus, T. flavus and R.

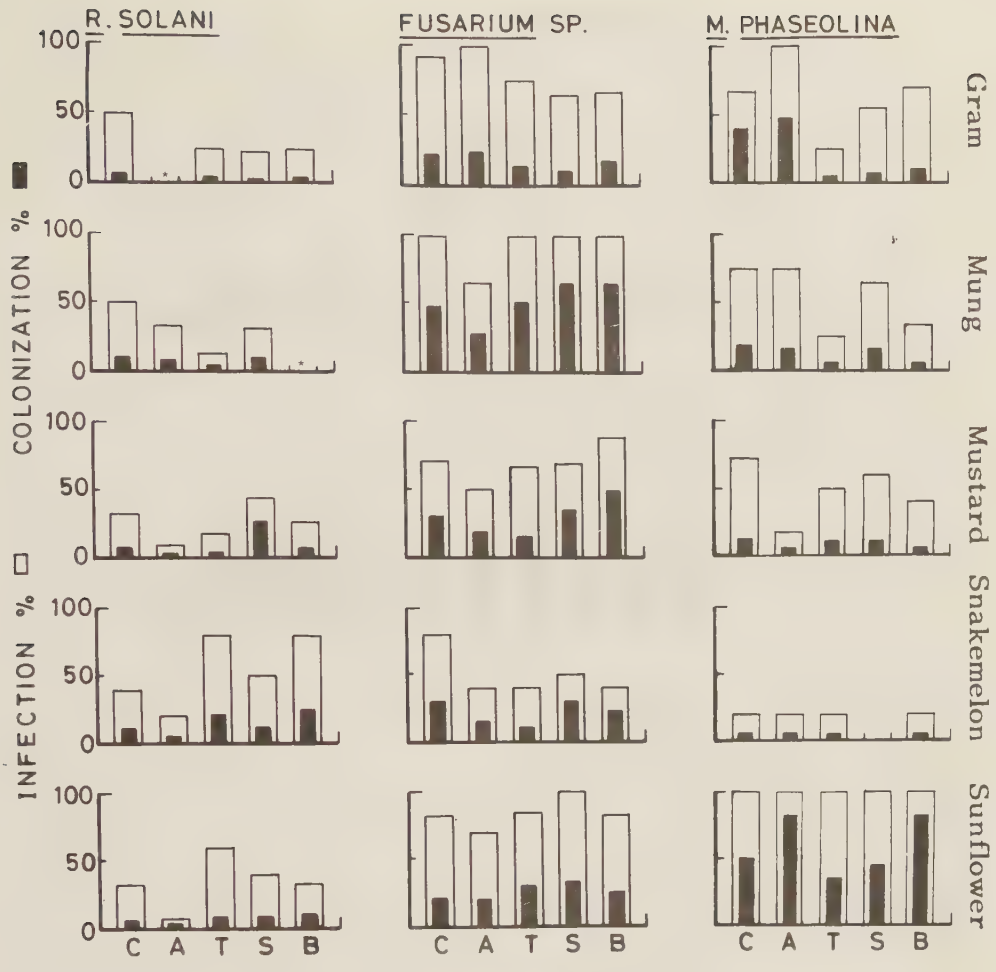

Fig. 12. Effect of seed dressing with microbial antagonists on the infection and colonization of gram, mung, mustard, snakemelon and sunflower roots by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp.

C= Control A= A. candidus T= T. harzianum S= Streptomyces sp. B= B. subtilis.

leguminosarum and 67% by A. candidus, T. hamatum, T. harzianum, Streptomyces sp., and R. meliloti. Similarly 34% reduction was observed where Arachniotus sp., and B. subtilis were used. Infection of roots by M. phaseolina reduced by upto 74% in T. harzianum and B. subtilis, 50% in A. candidus and Streptomyces sp., and 24% in T. hamatum, Arachniotus sp., and R. meliloti treatments (Fig. 13). T. flavus, P. lilacinus and R. leguminosarum were unable to

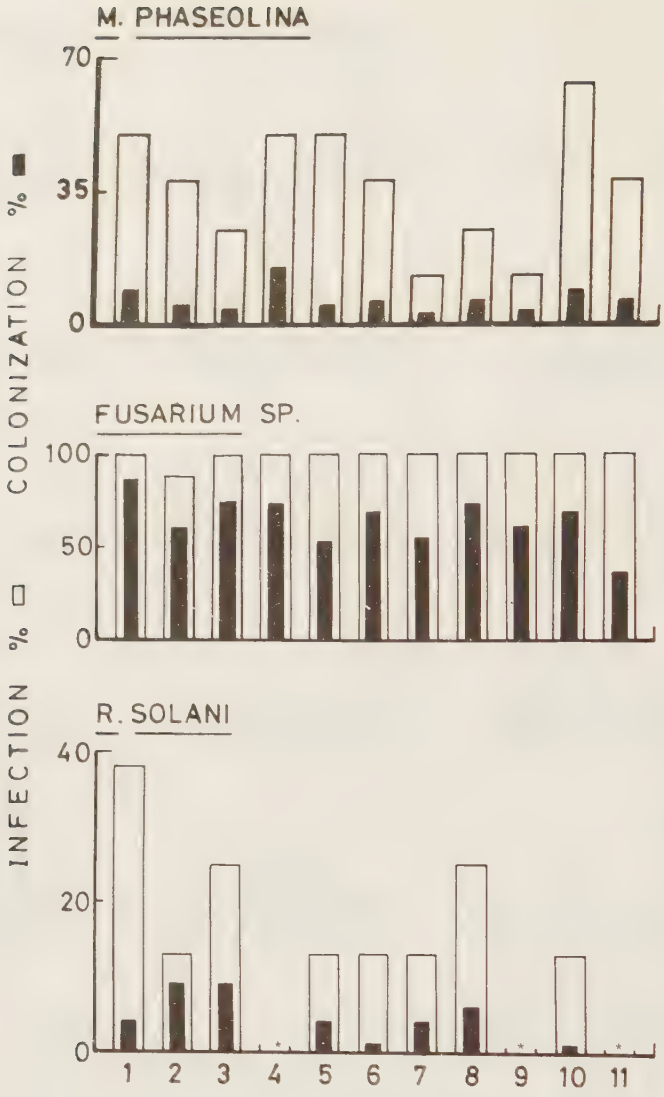

Fig. 13. Effect of seed dressing with microbial antagonists on infection and colonization of mung bean roots by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp.

1. Control 2. A. candidus 3. Arachniotus sp. 4. T. flavus 5. T. hamatum 6. T. harzianum 7. Streptomyces sp. 8. B. subtilis 9. R. leguminosarum 10. R. meliloti 11. P. lilacinus

reduce M. phaseolina infection on mung bean roots. It is interesting to note that infection of mung bean roots by R. solani and M. phaseolina was reduced by several of the test organisms as compared to Fusarium infection on test plants but frequency of colonization reduced by upto 15-58% by all the test organisms used (Fig. 13).

Experiments were repeated during 1987 where seeds of cotton (Gossypium arboreum L.) were treated with spore suspension of 7 day old cultures of microbial antagonists viz., Aspergillus candidus, Gliocladium virens, Paecilomyces lilacinus, Trichoderma harzianum and Streptomyces sp. Gum arabic (1% solution) was used as sticker. Seeds were sown in 1 x 3 m plots at the experimental field of the Department of Botany, University of Karachi during July, 1987. Each treatment was replicated 3 times and the treatments were randomized. After 60 days, plants were uprooted and the infection of roots by R. solani, M. phaseolina and Fusarium spp., was recorded.

Infection of cotton roots by R. solani reduced by upto 67% in G. virens, 50% in A. candidus, P. lilacinus and T. harzianum and, 41% in Streptomyces sp., treatments whereas infection of roots by M. phaseolina reduced only in A. candidus and P. lilacinus treatments where 100% reduction in infection was observed. Similarly P. lilacinus, A. candidus and Streptomyces sp., reduced Fusarium infection on cotton roots by upto 75,50 and 39% respectively (Fig. 14). G. virens and T. harzianum, however, failed to reduce Fusarium infection on cotton seedlings.

In another experiment carried out during May, 1987, at the experimental field of Cotton Research Institute, Sakrand, spore/cell suspension from 7 day old cultures of microbial antagonists viz., A. candidus, G. virens, P. lilacinus, T. harzianum, Bacillus subtilis and Rhizobium meliloti, were used for coating cotton seeds. Gum arabic (1% solution) was used as sticker. Each treatment was replicated 4 times with 3 rows in each replicate, and the treatments were randomized. Root infection by R. solani, M. phaseolina and Fusarium spp., was recorded after 30 days growth.

Use of microbial antagonists as seed treatment showed an increase in seed germination by 15-34% in all the treatments over the control (Fig. 15). Reduction in infection of roots by R. solani was 58% where G. virens and T. harzianum were used and 46, 37, 25 and 21% respectively, where P.

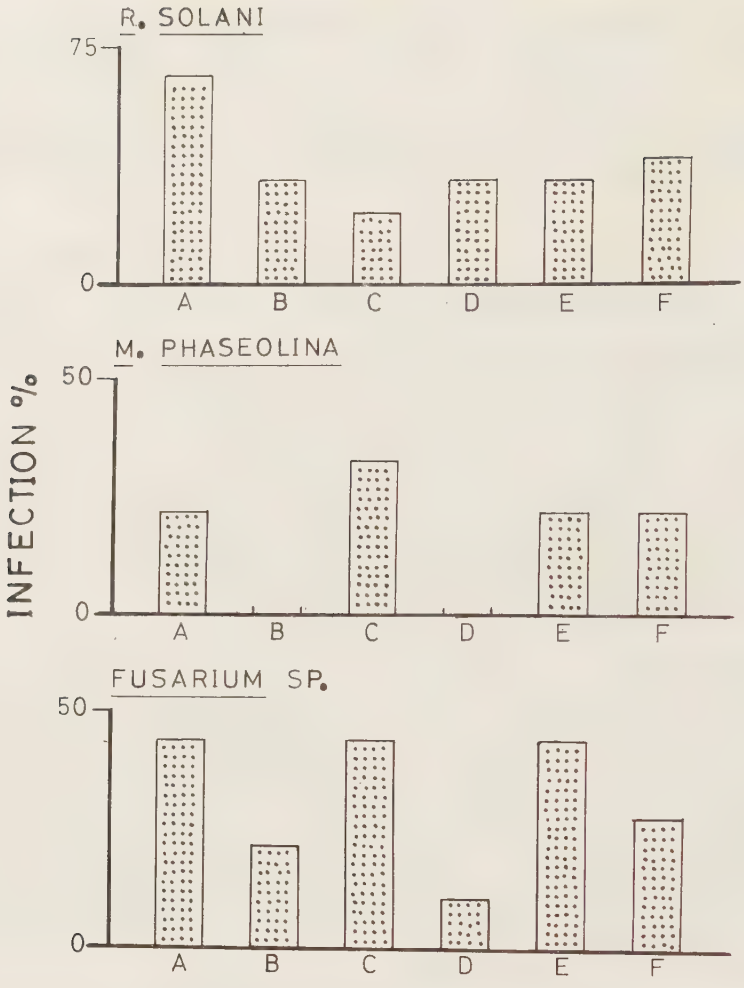

Fig. 14. Effect of seed dressing with microbial antagonists on root infection of cotton by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp. (Karachi, 1987).

A= Control B= A. candidus C= G. virens D= P. lilacinus E= T. harzianum F= Streptomyces sp.

lilacinus, B. subtilis, A. candidus and R. meliloti were used for seed treatment. Similarly infection of roots by M. phaseolina reduced by 100% in A. candidus, G. virens and P. lilacinus, and 50% in T. harzianum, B. subtilis and R. meliloti treatments. It is interesting to note that where fungi were ineffective in reducing infection by Fusarium sp., R. meliloti showed promising results where infection by Fusarium sp., was reduced by 17% (Fig. 15).

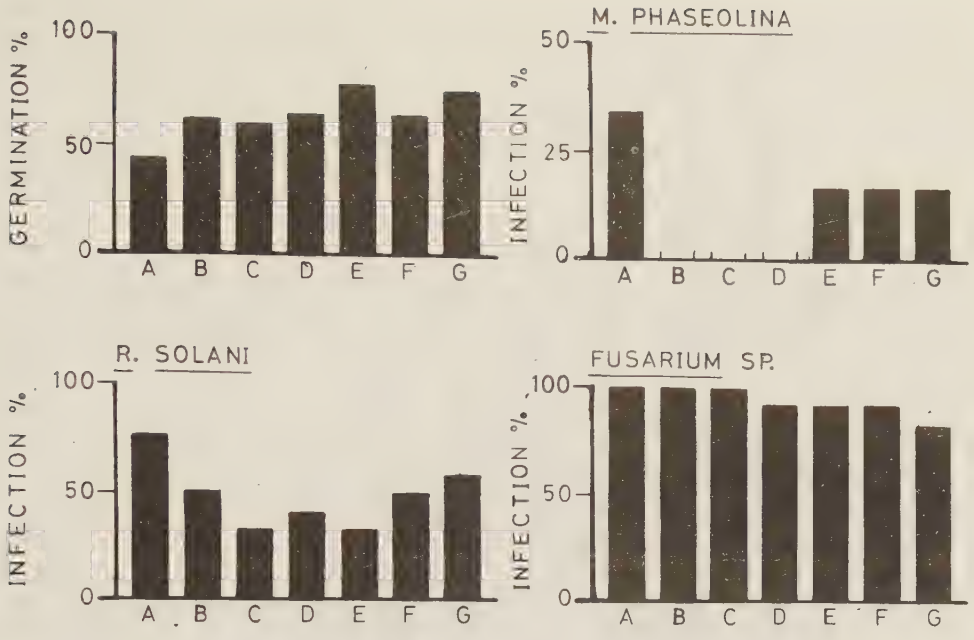

Fig. 15. Effect of seed dressing with microbial antagonists on germination and root infection of cotton by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp. (Sakrand, 1987).

A= Control **B**= A. candidus **C**= G. virens **D**= P. lilacinus **E**= T. harzianum **F**= B. subtilis **G**= R. meliloti

In another experiment bottle gourd (Lagenaria siceraria (Moll.) Standl.) was used as the test plant. Seeds of bottle gourd treated with microbial antagonists viz., A. candidus, G. virens, P. lilacinus, T. harzianum, Streptomyces sp., and R. meliloti were planted in field at the Department of Botany, University of Karachi, during May, 1987. R. solani infection on 30 day old plants reduced by upto 100% in G. virens, 20% in T. harzianum, 16% in P. lilacinus, 11% in A. candidus, R. meliloti, 11% in A. candidus and 7% in Streptomyces sp. Similarly M. phaseolina infection reduced by upto 40% in T. harzianum and Streptomyces sp., 33% in R. meliloti and A. candidus, 25% in P. lilacinus and G. virens treatments. Infection of roots by Fusarium spp.. reduced by upto 100% in Streptomyces sp., and R. meliloti, 47% in A. candidus, 11% in P. lilacinus and G. virens, 7% in T. harzianum. At harvest time, reduction in R. solani infection was 14, 46, 57, 62, 68 and 78% where seeds were treated with Streptomyces sp., R. meliloti, G. virens, A. candidus, P. lilacinus and T. harzianum respectively. M. phaseolina infection reduced

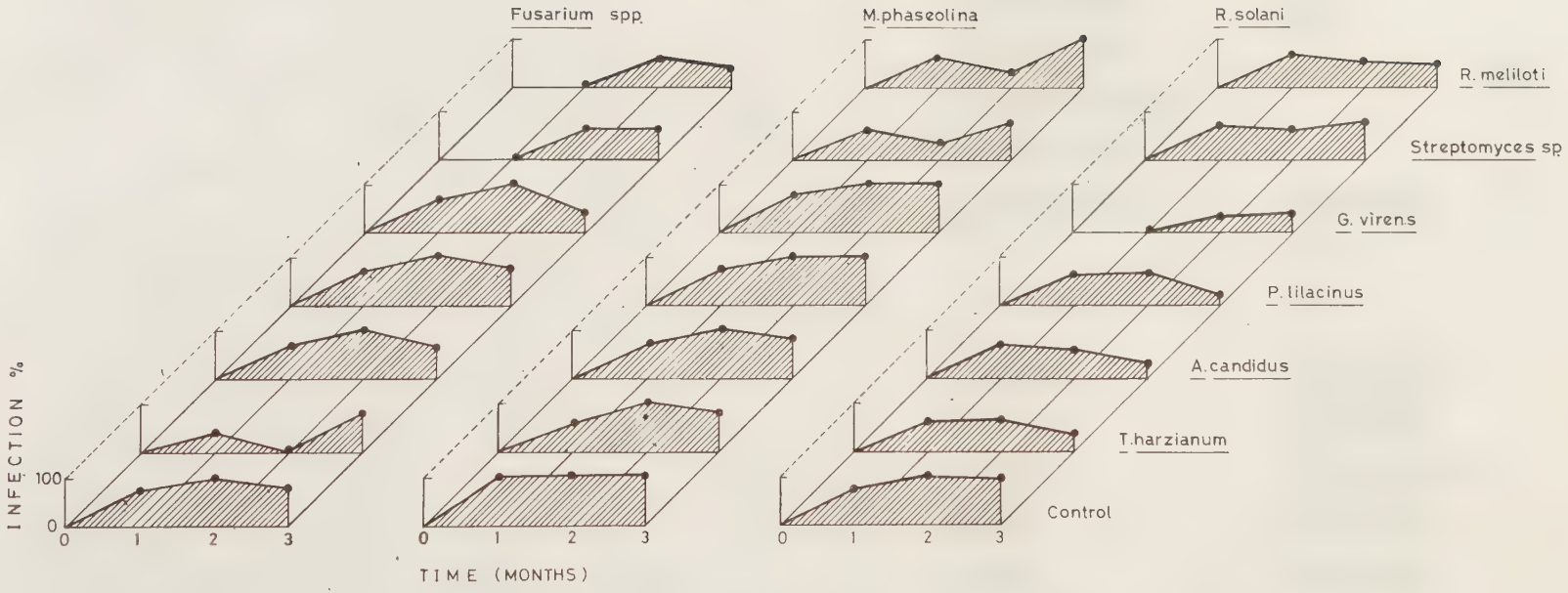

Fig. 16. Effect of seed dressing with microbial antagonists on the infection of bottle gourd roots by Macrophomina phaseolina, Rhizoctonia solani and Fusarium sp.

by upto 20% in T. harzianum, A. candidus and Streptomyces sp. In other treatments no reduction in M. phaseolina infection was observed. Root infection by Fusarium sp., reduced by upto 50% in R. meliloti and G. virens, 56% in A. candidus, 21% in P. lilacinus, 16% in Streptomyces sp., and 6% in T. harzianum (Fig. 16).

In another experiment carried out in May, 1987, okra seeds, after treatment with microbial antagonists viz., A. candidus, G. virens, P. lilacinus, T. hamatum, Streptomyces sp., B. subtilis and R. meliloti were planted in the field at Karachi University experimental plots. Observations recorded after 30 days showed that infection of roots by R. solani reduced by upto 63% (T. hamatum and B. subtilis) and 51% (G. virens, P. lilacinus, Streptomyces sp., and R. meliloti), whereas no reduction in Fusarium infection was observed. M. phaseolina infection reduced by 23% in T. hamatum treatment. R. solani infection on 60 days old plants reduced by upto 100% in G. virens and T. hamatum, 51% in P. lilacinus, B. subtilis and R. meliloti and 20% in A. candidus treatments. Similarly reduction in M. phaseolina infection was 22% in A. candidus, 28% in G. virens, 50% in P. lilacinus, 16% in T. hamatum and B. subtilis and 33% in R. meliloti treatments. Fusarium infection reduced by upto 25, 33 and 75%, respectively, in treatments where P. lilacinus, G. virens and R. meliloti were used. (Fig. 17).

In another experiment carried out during February, 1988, treatment of mung bean seeds with microbial antagonists viz., A. candidus, G. virens, P. lilacinus, T. harzianum and R. leguminosarum reduced R. solani infection on 30 days old seedlings respectively by upto 20, 30, 30, 20 and 40%, whereas, infection of roots by M. phaseolina reduced by upto 9, 16, 24, 34 and 46% where respectively G. virens, T. harzianum, R. leguminosarum, P. lilacinus and A. candidus were used (Fig. 18). Fusarium infection was not reduced in any of the treatments.

3. Use of antagonists grown on wheat straw

Soil amendment with microbial antagonists can decrease rapid saprophytic development of the pathogen during early stages of soil recolonization which may reduce the infection and disease severity (Marois et al, 1981). Actively growing hyphae of Trichoderma spp., and G. virens in bran culture have been found more effective against R. solani as compared to conidial preparations (Lewis & Papavizas, 1985). Effect of soil amendment with antagonists in mycelial state was therefore examined.

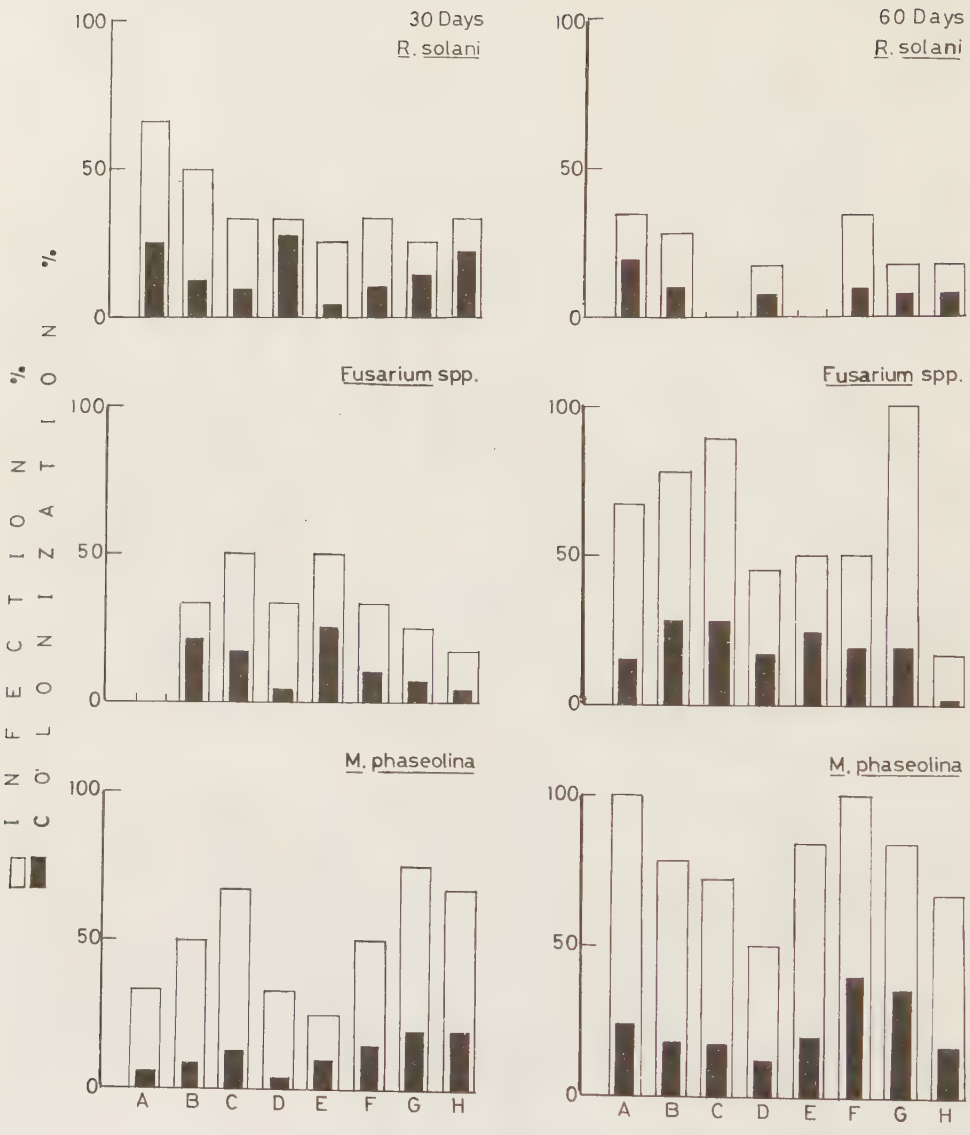

Fig. 17. Effect of seed coating with antagonistic microorganisms on infection of okra roots by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp.

A= Control B= A. candidus C= G. virens D= P. lilacinus E= T. hamatum F= Streptomyces sp. G= B. subtilis H= R. meliloti

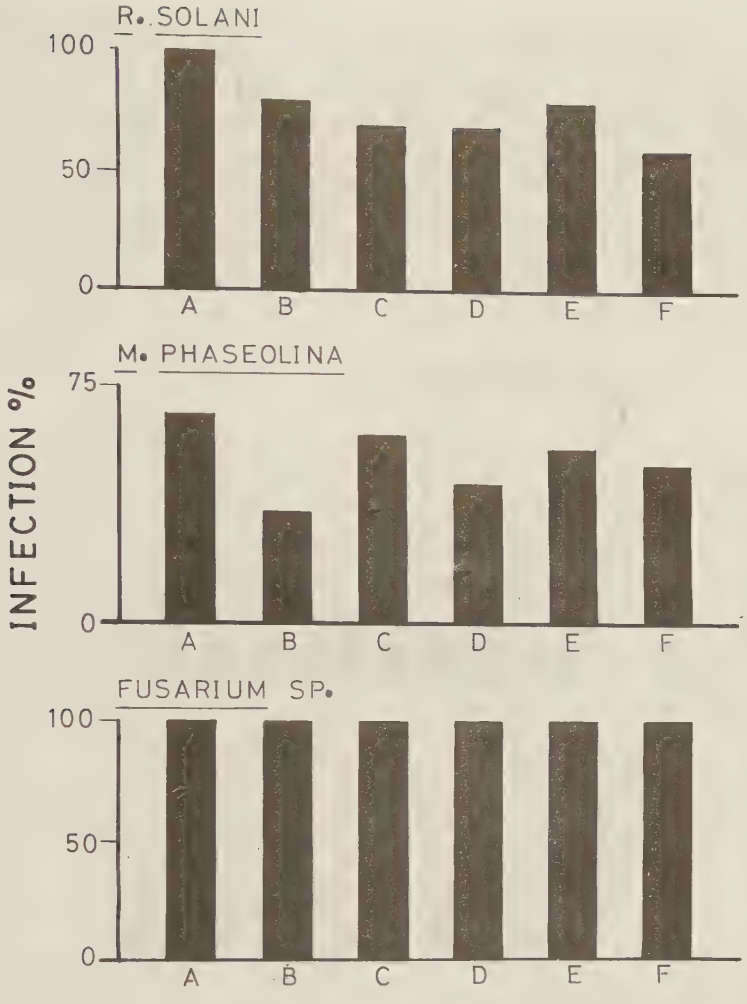

Fig. 18. Effect of seed dressing with microbial antagonists on infection of mung bean roots by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp.

A= Control **B**= A. candidus **C**= G. virens **D**= P. lilacinus **E**= T. harzianum **F**= R. leguminosarum

Microbial antagonists viz., Arachniotus sp., A. candidus, P. lilacinus, T. flavus, T. hamatum, T. harzianum, Streptomyces sp., B. subtilis, R. leguminosarum and R. meliloti were multiplied on wheat straw for 3 days and applied to the field in furrows at the time of planting mung bean seeds during May, 1986. Root samples were collected after a growth period of 30 days and percentage infection and colonization of roots by R. solani, M. phaseolina and Fusarium spp., was assessed.

Whereas Streptomyces sp., reduced R. solani infection by 27%, all other test organisms were unable to protect mung bean roots from R. solani infection. Infection of roots by M. phaseolina reduced in P. lilacinus (86%), Sreptomyces sp., (69%), R. legumonisarum (43%), A. candidus (29%), B. subtilis (29%), T. hamatum (14%) and Arachniotus sp., (14%). Similarly reduction in Fusarium infection was 41, 38 and 28% where respectively T. flavus, T. hamatum and T. harzianum were used (Fig. 19).

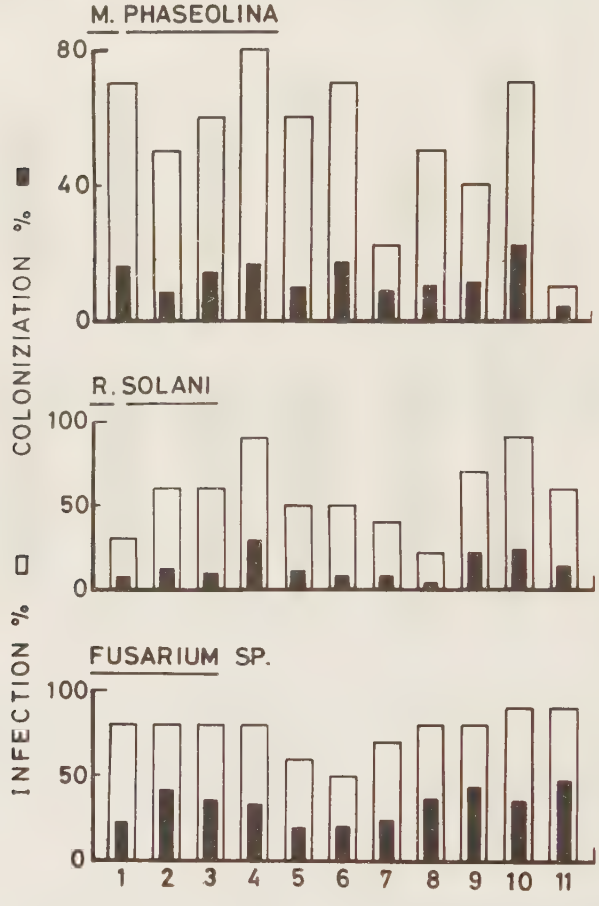

Fig. 19. Effect of microbial antagonists (grown on wheat straw) on infection and colonization of mung bean roots by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp.

1.= Control 2.= Arachniotus sp. 3.= A. candidus 4.= P. lilacinus 5.= T. flavus 6.= T. hamatum 7.= T. harzianum 8.= Streptomyces sp. 9.= B. subtilis 10.= R. leguminosarum 11.= R. meliloti

The effectiveness of soil treatment with microbial antagonists was further tested during 1987. In an experiment, carried out at the experimental field of Cotton Research Institute, Sakrand, spore/cell suspensions of microbial antagonists viz., A. candidus, G. virens, P. lilacinus, T. harzianum, B. subtilis and R. meliloti were separately inoculated on wheat straw and incubated for 12 h before their application in the field in furrows at the time of sowing cotton seeds. Each treatment was replicated 4 times with 3 rows in each treatment and the treatments were randomized. Root infection by R. solani, M. phaseolina and Fusarium spp., was recorded after 30 days growth of cotton.

Germination of cotton seeds showed an increase by 5-11% in all the treatments except A. candidus and B. subtilis where germination reduced by 7 and 8% respectively (Fig. 20). Infection of roots by R. solani decreased by upto 50% in T. harzianum, 30% in A. candidus and P. lilacinus and, 20% in G. virens and B. subtilis treatments. Similarly M. phaseolina infection reduced by 40% in A. candidus, T. harzianum, B. subtilis and R. meliloti, and 20% in P. lilacinus treatment. Soil application of G. virens failed to reduce M. phaseolina infection. A. candidus and B. subtilis reduced Fusarium infection by 10%, whereas, G. virens, P. lilacinus, T. harzianum and R. meliloti were found ineffective in reducing Fusarium infection on cotton roots (Fig. 20).

4. Effect of culture filtrate of microbial antagonists

Inhibition of growth of pathogens by an antagonist is generally referred as antibiosis since microorganisms produce toxic metabolites inhibitory to growth of other microorganisms. An experiment was carried out at the experimental field of the Department of Botany, University of Karachi to study the effect of antibiotics produced by antagonistic microorganisms during their cultivation on liquid media.

Microbial antagonists viz., A. candidus, G. virens, P. lilacinus and T. harzianum were grown on Potato Sucrose broth for 15 days at room temperature and the culture filtrates were used for treatment of soybean (Glycine max (L.) Merr.) and wheat (Triticum aestivum L.) seeds. Seeds were sown during January, 1988, and infection of roots by root infecting fungi was recorded after 30 days growth.

In soybean, infection of roots by R. solani reduced by 80, 66, 65 and 34%, respectively, in T. harzianum, A.

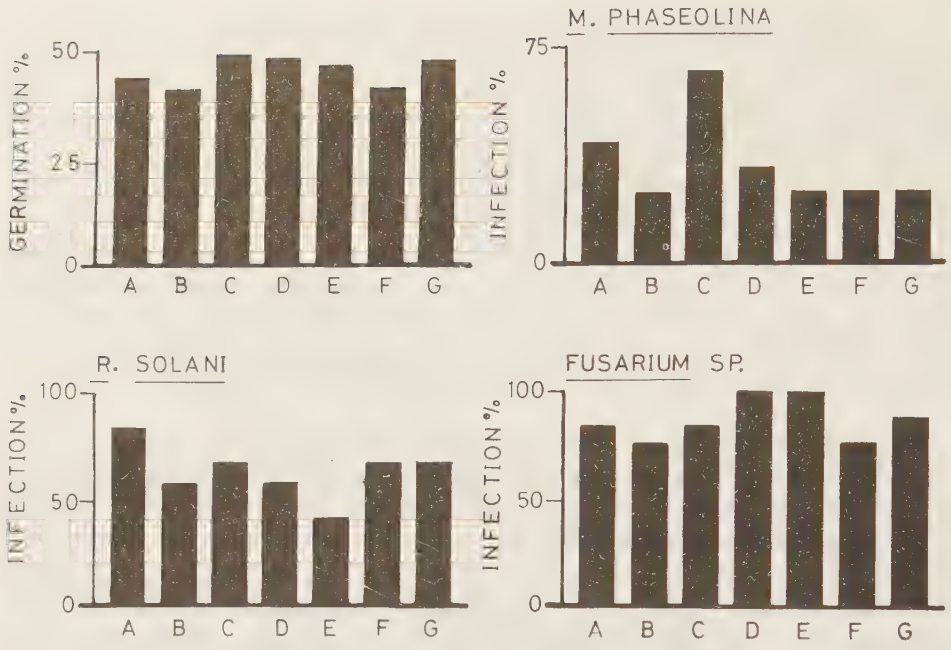

Fig. 20. Effect of soil treatment with microbial antagonists (grown on wheat straw) on germination of cotton seeds and infection of cotton roots by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp.

A= Control B= A. candidus C= G. virens D= P. lilacinus E= T. harzianum F= B. subtilis G= R. meliloti

candidus, G. virens and P. lilacinus treatments, whereas, M. phaseolina infection reduced by 65, 35, 100 and 60% respectively. Fusarium infection was only reduced by 17% in G. virens treatment (Fig. 21). In wheat, R. solani infection reduced by 42, 22, 7 and 0%, and M. phaseolina infection by 35, 65, 100 and 100% respectively in P. lilacinus, G. virens, A. candidus and T. harzianum treatments, whereas, Fusarium infection only reduced by 13% where culture filtrate of A. candidus was used (Fig. 21).

5. Delivery of antagonists

Jager and Velvis (1985) reported that inoculation of seed potatoes with microbial antagonists was effective in reducing R. solani infection on potato, whereas, other workers viz., Venkatasublaiah and Safeeulla (1984), Venkatasublaiah et al (1984) and Lewis and Papavizas (1985) found that incorporation of actively growing culture of microbial anta-

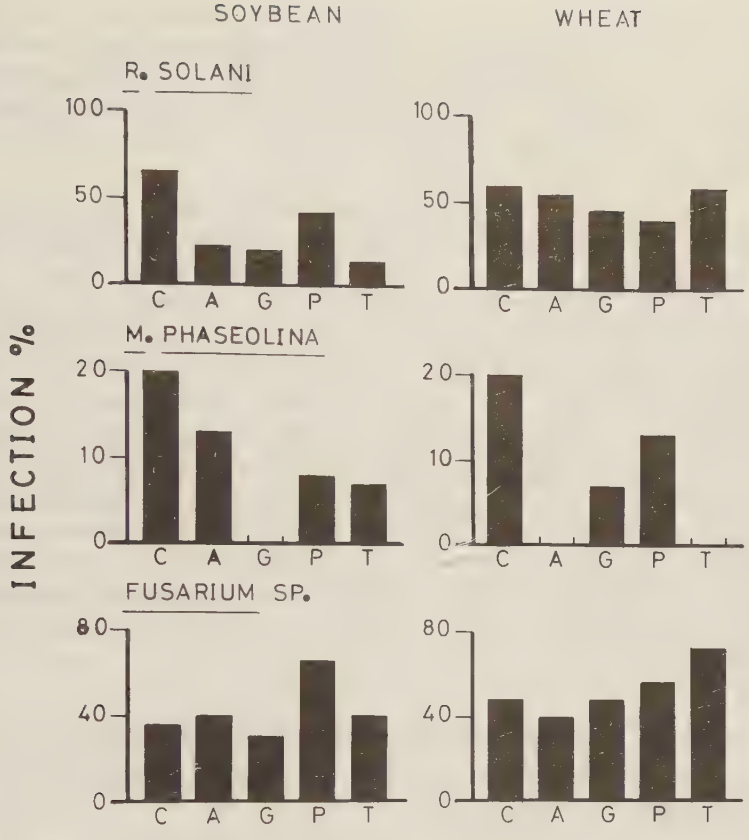

Fig. 21. Effect of seed treatment with culture filtrate of microbial antagonists on root infection of soybean and wheat by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp.

C= Control A= A. candidus G= G. virens P= P. lilacinus T= T. harzianum

gonists was superior to the seed treatment method. Experiments were, therefore, carried out to find out the effective means for the delivery of antagonists in soil.

Soil artificially infested with R. solani (@ 1% w/w) was kept in 8 cm diameter plastic pots. Microbial antagonists viz., A. candidus, G. virens, P. lilacinus and T. harzianum were used either as seed dressing or incorporated into soil (@ 1% w/w) after growing on wheat straw, rice straw, rice grains and sorghum grains, for 3 weeks at room temperature. Seeds of okra were sown in March, 1987, and after 30 days growth the plants were uprooted to assess root infection by R. solani.

Germination of seeds showed an increase in all treatments (11-47%) but highest germination was observed where antagonists were used alongwith rice grains followed by seed dressing or use of inoculum multiplied on wheat straw, rice straw or sorghum grains (Table 6).

P. lilacinus was more effective in reducing R. solani infection on okra when used as seed dressing (81%) followed by its application on rice grains (63%), rice straw (37%), sorghum grains (25%) and wheat straw (21%). A. candidus was found most effective when used alongwith rice straw, in reducing R. solani infection by 44% followed by seed dressing where 25% reduction in infection was observed. Similarly, reduction in R. solani infection by T. harzianum was highest when used with sorghum grains (38%) followed by seed dressing (36%) and rice straw or rice grains (25%) whereas G. virens was found most effective when used alongwith rice grains, in reducing R. solani infection by 63%, followed by rice straw (51%), wheat straw and sorghum grains (38%) and seed dressing where 29% reduction in infection was observed (Table 6).

Table 6. EFFECT OF SEED DRESSING AND DIFFERENT SUBSTRATES FOR THE GROWTH OF MICROBIAL ANTAGONISTS AND THEIR FIELD APPLICATION FOR THE CONTROL OF RHIZOCTONIA SOLANI INFECTION ON OKRA.

1. GERMINATION %

TREATMENTS	Control	Microbial Antagonists			
		A. candidus	G. virens	P. lilacinus	T. harzianum
Seed dressing	63	80	87	87	80
Wheat straw	63	80	87	87	80
Rice straw	63	80	73	83	83
Rice grains	63	83	93	87	87
Sorghum grains	63	73	80	80	

2. R. SOLANI INFECTION %

TREATMENTS	Control	Microbial Antagonists			
		A. candidus	G. virens	P. lilacinus	T. harzianum
Seed dressing	89	67	63	17	57
Wheat straw	89	67	55	78	89
Rice straw	89	50	44	56	67
Rice grains	89	89	33	33	67
Sorghum grains	89	89	55	67	55

In a field experiment during March, 1987, at the Department of Botany, University of Karachi, microbial antagonists viz., A. candidus, G. virens, P. lilacinus and T. harzianum were used either as seed dressing or applied to soil in furrows @ 40 g/m after growing them on wheat straw, rice straw, rice grains or sorghum grains for 3 weeks at room temperature. Okra was used as a test plant and infection of roots by R. solani was recorded after 30 days growth.

Germination of seeds was highest where antagonists were used alongwith rice grains followed by seed dressing (Table 7). The results indicate that the use of rice grains as a substrate for microbial antagonists viz., A. candidus, G. virens, P. lilacinus and T. harzianum was most effective in reducing root infection by R. solani (30-50%) followed by sorghum grains (20-35%) or coating seeds with microbial antagonists in which 20-30% reduction in R. solani infection was obsrved (Table 7). Similarly, highest reduction in M. phaseolina infection was observed where A. candidus and P. lilacinus were used on rice grains (75 and 70% respectively) or G. virens and T. harzianum were used on sorghum grains (80 and 65% respectively). Use of wheat straw or rice straw as a substrate for microbial antagonists or coating seeds with microbial antagonists were found comparatively less effective in reducing M. phaseolina infection on okra. A. candidus, G.virens and T. harzianum were found most effective against Fusarium sp., where seeds were coated with spore suspension, reducing root infection by 11, 100 and 18% respectively, whereas, P. lilacinus when used on rice grains reduced Fusarium infection by 100% (Table 7).

d. Effect of organic fertilizers

Soil fertility may influence the incidence and severity of root diseases. The effect varies with the pathogen and the fertilizer used since an appropriate change in soil fertility can substantially reduce injury by soilborne pathogens (Millner et al, 1982; Hoitink, 1980; Van Assche & Uyttebroeck, 1981; Ghaffar, 1987). Experiments were therefore carried out to study the effect of soil amendment with different organic fertilizers on the reduction in infection by soilborne root infecting fungi.

Expt.i. At the experimental field of the Department of Botany, University of Karachi, soil was amended with organic fertilizers viz., cow dung, sewage sludge and Zarkhez @ 1% w/w during July, 1987. Cotton seeds were sown in randomized

Table 7. EFFECT OF SEED DRESSING AND DIFFERENT SUBSTRATES FOR THE GROWTH OF MICROBIAL ANTAGONISTS AND THEIR FIELD APPLICATION FOR THE CONTROL OF ROOT INFECTION BY *RHIZOCTONIA SOLANI*, *MACROPHOMINA PHASEOLINA* AND *FUSARIUM* SP.

1. GERMINATION %

		Microbial Antagonists			
TREATMENTS	Control	*A. candidus*	*G. virens*	*P. lilacinus*	*T. harzianum*
Seed dressing	49	58	56	62	60
Wheat straw	49	44	52	66	56
Rice straw	49	38	62	44	40
Rice grains	49	56	82	70	60
Sorghum grains	49	32	50	54	44

2. *R. SOLANI* INFECTION %

		Microbial Antagonists			
TREATMENTS	Control	*A. candidus*	*G. virens*	*P. lilacinus*	*T. harzianum*
Seed dressing	100	70	80	70	67
Wheat straw	100	90	80	80	80
Rice straw	100	100	70	80	80
Rice grains	100	50	70	50	70
Sorghum grains	100	80	80	65	75

3. *M. PHASEOLINA* INFECTION %

		Microbial Antagonists			
TREATMENTS	Control	*A. candidus*	*G. virens*	*P. lilacinus*	*T. harzianum*
Seed dressing	100	60	30	80	47
Wheat straw	100	57	70	50	40
Rice straw	100	75	70	80	60
Rice grains	100	25	50	30	40
Sorghum grains	100	80	80	65	75

4. *FUSARIUM* INFECTION%

		Microbial Antagonists			
TREATMENTS	Control	*A. candidus*	*G. virens*	*P. lilacinus*	*T. harzianum*
Seed dressing	24	20	0	10	20
Wheat straw	24	90	70	20	30
Rice straw	24	58	40	40	33
Rice grains	24	50	50	0	30
Sorghum grains	24	70	20	90	48

block design with 3 replicates of each treatment. After 60 days growth plants were uprooted and infection of roots by R. solani, M. phaseolina and Fusarium spp., was recorded.

In plots amended with cow dung, cotton plants showed 67% less R. solani infection as compared to plants grown in nonamended plots. Reduction in R. solani infection was 50% where soil was amended with either sewage sludge or Zarkhez. None of the fertilizers used reduced M. phaseolina infection whereas Fusarium infection was only reduced by 50% where soil was amended with Zarkhez (Fig. 22).

Expt.ii. In another experiment soil artificially infested with R. solani @ 1% w/w was amended with organic fertilizers viz., cow dung, buffalo dung, goat dung, sewage sludge, chicken manure, Ravi and Zarkhez @ 5% w/w each. Soil was kept in 7 cm diameter plastic pots and seeds of bottle gourd sown at 0-time and after 5, 10 and 20 days of treatment. Soil moisture was adjusted and maintained at 50% MHC. After 30 days where the plants were sown at 0-time of treatment showed upto 42% reduction in R. solani infection in Ravi, 20% in sewage sludge and chicken manure, 11% in Zarkhez and 7% in buffalo dung and goat dung amendments whereas no reduction in infection was observed in soil amended with cow dung. Plants sown after 5 days of treatment showed no significant reduction in R. solani infection whereas in plants

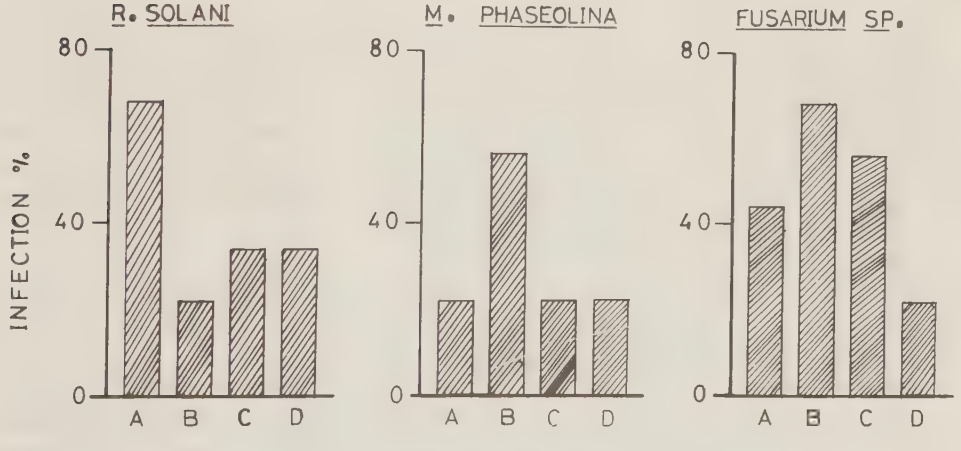

Fig. 22. Effect of organic fertilizers on infection of cotton roots by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp.

A= Control **B**= Cow dung **C**= Sewage sludge **D**= Zarkhez

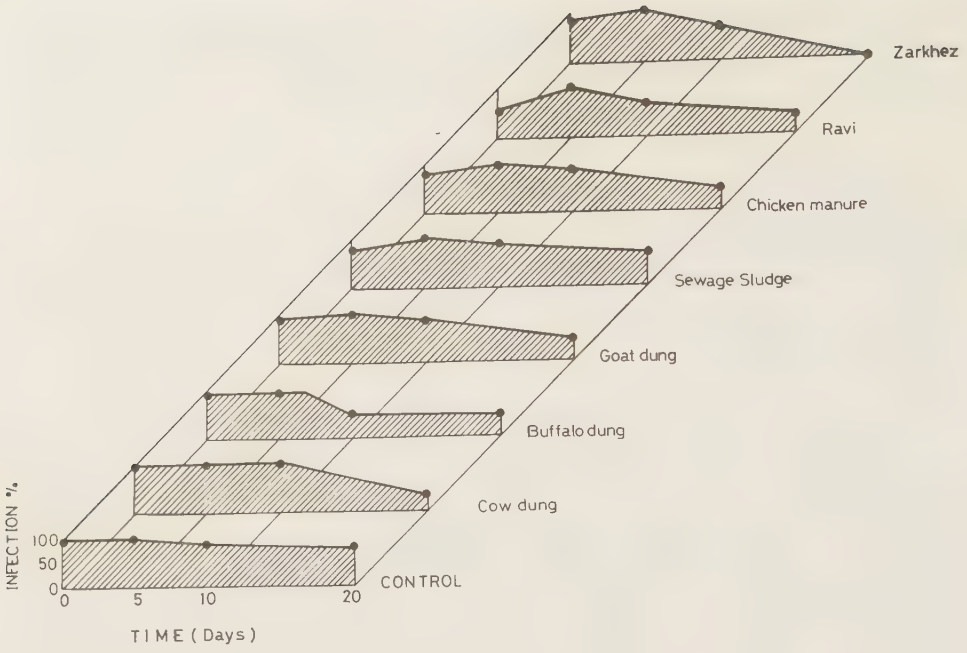

Fig. 23. Effect of fertilizers on infection of bottle gourd roots by *Rhizoctonia solani*.

sown after 20 days of amendment, reduction in *R. solani* infection was 100% in Zarkhez, 58% in cow dung, 44% in chicken manure and Ravi Pak, 42% in buffalo dung and goat dung and 14% in sewage sludge amended soil (Fig. 23).

Expt.iii. In a pot experiment where soil artificially infested with *R. solani* @ 1% w/w, was amended with cow dung, sewage sludge, Ravi, Farooq or Zarkhez @ 1% w/w, germination of okra seeds increased upto 15% in Ravi and Farooq, 25% in sewage sludge and Zarkhez and 50% in cow dung amended soils. Infection of roots by *R. solani* reduced by 25, 38, 50 and 86% in soils amended with Zarkhez, Farooq, sewage sludge and cow dung respectively (Fig. 24).

Expt.iv. The experiment was repeated in February, 1988, where seeds of mung bean were sown in microplots (1X3 m) supplemented with different organic fertilizers viz., cow dung, sewage sludge and Zarkhez (@ 1% w/w). After 30 days growth infection of roots by *R. solani* reduced by upto 50, 47 and 36% respectively in treatments where Zarkhez, cow dung and sewage sludge were used whereas no noticeable reduction in *M. phaseolina* or *Fusarium* infection was observed in any of the treatments (Fig. 25).

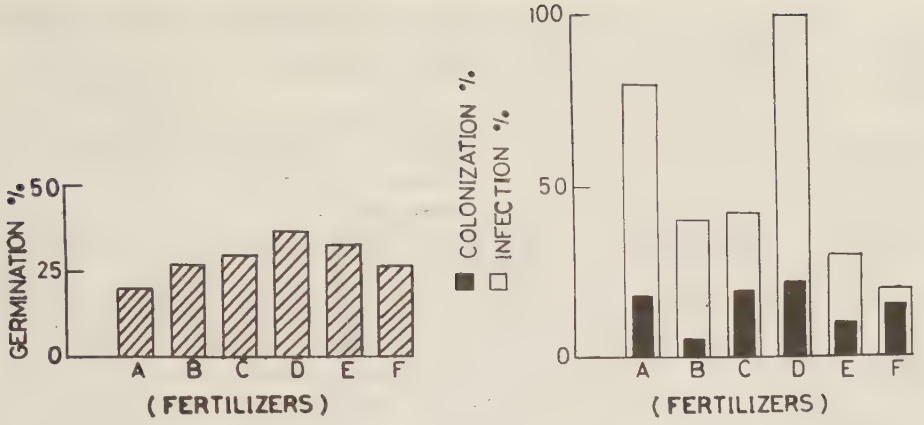

Fig. 24. Effect of organic fertilizers on seed germination, infection and colonization of okra roots by Rhizoctonia solani.

A= Control **B**= Sewage sludge **C**= Cow dung **D**= Ravi **E**= Farooq **F**= Zarkhez

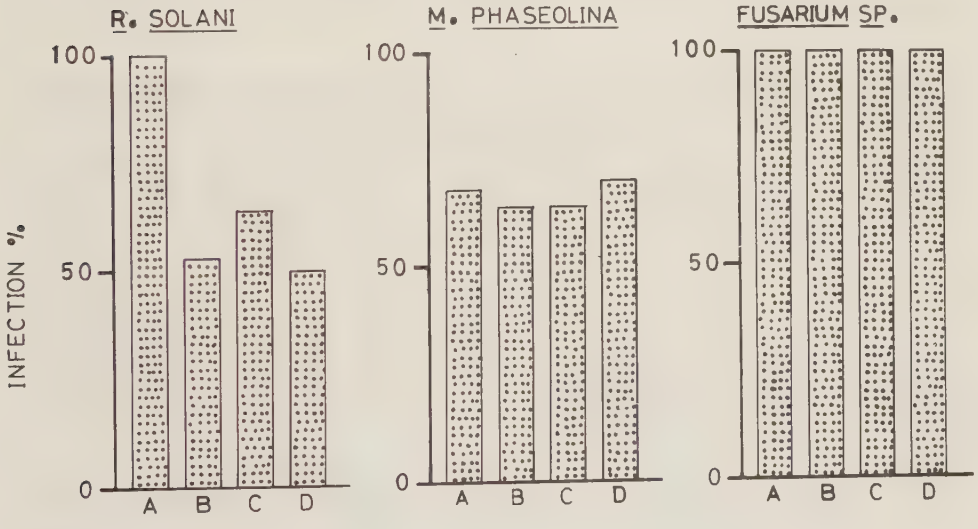

Fig. 25. Effect of organic fertilizers on infection of mung bean root by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp.

A= Control **B**= Cow dung **C**= Sewage sludge **D**= Zarkhez

e. Combined effect of organic fertilizers and microbial antagonists

From the results of the experiments reported it would appear that control of plant diseases is rarely achieved by a single procedure because different methods work best at different times and at different places, or under varying conditions. Baker and Cook (1974) have ,therefore, suggested to integrate more than one methods of control. In our experiments organic fertilizers and microbial antagonists have been found to effectively reduce infection of roots by soilborne fungi. Experiments were, therefore, carried out to study the combined effect of organic fertilizers and microbial antagonists on infection of roots by root infecting fungi.

Expt.i. Natural soil at the experimental plots of the Department of Botany, University of Karachi, was amended with organic fertilizers viz., cow dung, sewage sludge and Zarkhez @ 1% w/w in July, 1987. Cotton seeds were treated with spore suspension of microbial antagonists viz., A. candidus, G. virens, P. lilacinus, T. harzianum and Streptomyces sp., and planted in field. Non-treated seeds in non-amended soil served as control. Infection of roots by root infecting fungi was assessed after 30 days growth of plants.

An integrated use of microbial antagonists and organic fertilizers was found more effective in the control of root infecting fungi as compared to microbial antagonists or organic fertilizers used alone. Infection of root by R.solani was completely inhibited in all treatments where antagonists were used with fertilizers except where T. harzianum was used with sewage sludge or Streptomyces sp., was used with cow dung which resulted in upto 83% reduction in R. solani infection over the control plots (Table 8). Similarly significant reduction in M. phaseolina infection (100%) was observed in treatments where Streptomyces sp., was used with Zarkhez whereas infection increased in all the other treatments. Similarly greater reduction in Fusarium infection was observed in treatments where microbial antagonists and organic fertilizers were integrated as compared to their use alone (Table 8).

Expt.ii. A similar experiment was carried out during February, 1988, where mung bean was used as test plant. Infection of mung bean roots by R. solani significantly reduced in treatments where P. lilacinus was used with cow dung. Similarly combined use of T. harzianum or R. leguminosarum

Table 8. COMBINED EFFECT OF MICROBIAL ANTAGONISTS AND ORGANIC FERTILIZERS ON INFECTION OF MUNG ROOTS BY RHIZOCTONIA SOLANI, MACROPHOMINA PHASEOLINS AND FUSARIUM SP.

1. R. SOLANI INFECTION %

Antagonists	Organic fertilizers			
	Control	Cow dung	Sewage sludge	Zarkhez
Control	67	22	33	33
A. candidus	33	0	0	0
G. virens	22	0	0	0
P. lilacinus	33	0	0	0
T. harzianum	33	0	11	0
Streptomyces sp.	39	11	0	0

2. M. PHASEOLINA INFECTION %

Antagonists	Organic fertilizers			
	Control	Cow dung	Sewage sludge	Zarkhez
Control	22	56	22	22
A. candidus	0	50	33	44
G. virens	33	33	28	33
P. lilacinus	0	44	33	44
T. harzianum	22	55	55	33
Streptomyces sp.	22	25	33	0

3. FUSARIUM INFECTION %

Antagonists	Organic fertilizers			
	Control	Cow dung	Sewage sludge	Zarkhez
Control	44	67	56	22
A. candidus	22	11	0	11
G. virens	44	11	0	0
P. lilacinus	11	0	11	22
T. harzianum	44	33	0	0
Streptomyces sp.	28	11	11	11

with Zarkhez showed greater reduction of M. phaseolina infection than their use alone. No marked reduction in Fusarium infection was observed in any of the treatments (Table 9).

Expt.iii. In another experiment P. lilacinus was grown on rice grains. Fifteen day old rice grain culture of P. lilacinus was added to soil @ 1% w/w and the soil was amended with organic fertilizers viz., cow dung, sewage sludge, Zarkhez or Ravi @ 1% w/w. Soil samples in nylon bags were buried in field and at 0-time and after 10, 20 and 40 days of

Table. 9. COMBINED EFFECT OF MICROBIAL ANTAGONISTS AND ORGANIC FERTILIZERS ON INFECTION OF MUNG ROOTS BY RHIZOCTONIA SOLANI, MACROPHOMINA PHASEOLINA AND FUSARIUM SP.

1. R. SOLANI INFECTION %

Antagonists	Organic fertilizers			
	Control	Cow dung	Sewage sludge	Zarkhez
Control	100	53	64	50
A. candidus	80	69	83	100
G. virens	70	89	83	100
P. lilacinus	70	78	58	92
T. harzianum	80	75	78	78
R. leguminosarum	60	53	67	61

2. M. PHASEOLINA INFECTION %

Antagonists	Organic fertilizers			
	Control	Cow dung	Sewage sludge	Zarkhez
Control	67	64	64	67
A. candidus	36	53	67	69
G. virens	61	92	58	72
P. lilacinus	44	78	64	61
T. harzianum	56	61	64	33
R. leguminosarum	51	72	50	42

3. FUSARIUM INFECTION %

Antagonists	Organic fertilizers			
	Control	Cow dung	Sewage sludge	Zarkhez
Control	100	100	100	100
A. candidus	100	100	100	100
G. virens	100	92	100	92
P. lilacinus	100	100	100	100
T. harzianum	100	100	100	100
R. leguminosarum	100	100	100	100

amendment the bags were removed and soil transferred into 8 cm diameter plastic pots. Mung bean @ 10 seed/pot was sown and infection of roots by root infecting fungi was assessed after 30 days of growth.

In plants sown at 0-time of treatment, infection of roots by R. solani reduced by 21% where P. lilacinus was used alone, and by 100, 77, 34 and 0% where P. lilacinus was used with Ravi, cow dung, sewage sludge and Zarkhez,

respectively. Highest reduction in R. solani infection was observed in plants sown after 20 days of treatment where P. lilacinus was used alone or mixed with cow dung, sewage sludge and Zarkhez. Infection of roots by R. solani however increased in all the treatments after 40 days (Fig. 26). Similarly M. phaseolina infection in plants sown at 0-time decreased, respectively, by upto 61, 39, 39, 32 and 32% in treatments where P. lilacinus was used alone or mixed with cow dung, sewage sludge, Zarkhez and Ravi. In plants sown after 40 days of treatment, reduction in M. phaseolina infection was observed only in treatments where P. lilacinus was used alone (36%) or mixed with sewage sludge (30%). Infection of roots by Fusarium spp., in plants sown at 0-time was greater in P. lilacinus treatment as compared to control but infection decreased by upto 68, 59 and 59% in treatments where P. lilacinus was used with cow dung, sewage sludge and Zarkhez respectively. In plants sown after 10 days of treatment, Fusarium infection respectively reduced by 25, 72, 56, 31 and 20% over the control where P. lilacinus was used alone or mixed with cow dung, Zarkhez, sewage sludge or Ravi, whereas in plants sown after 40 days of treatment, reduction in Fusarium infection (16%) was observed only where P. lilacinus was used with sewage sludge (Fig. 26).

Greater reduction in infection of roots by R. solani, M. phaseolina and Fusarium spp., was observed in plants sown at 0-time or after 10 and 20 days of treatment. The effect of P. lilacinus and fertilizers started to decline after 20 days. Combined use of P. lilacinus and fertilizers gave better results against R. solani and Fusarium than those of P. lilacinus alone. P. lilacinus alone was found more effective against M. phaseolina than its use alongwith fertilizers.

f. Effect of organic amendment

Reports have been made where organic matter added to soil may increase, decrease or may not affect diseases caused by soilborne plant pathogens (Lumsden et al, 1983; Papavizas & Lumsden, 1980). Experiments were, therefore, carried out to study the effect of different organic substrates on root infecting fungi.

Expt.i. Soil artificially infested with sclerotia of M. phaseolina @ 30 scl./g of soil was kept in 8 cm diameter plastic pots. The soil was amended with molasses or with dried, ground wheat straw or lucerne @ 1 and 10% w/w. Non-amended soil served as control. Soil samples were removed at 0-time and

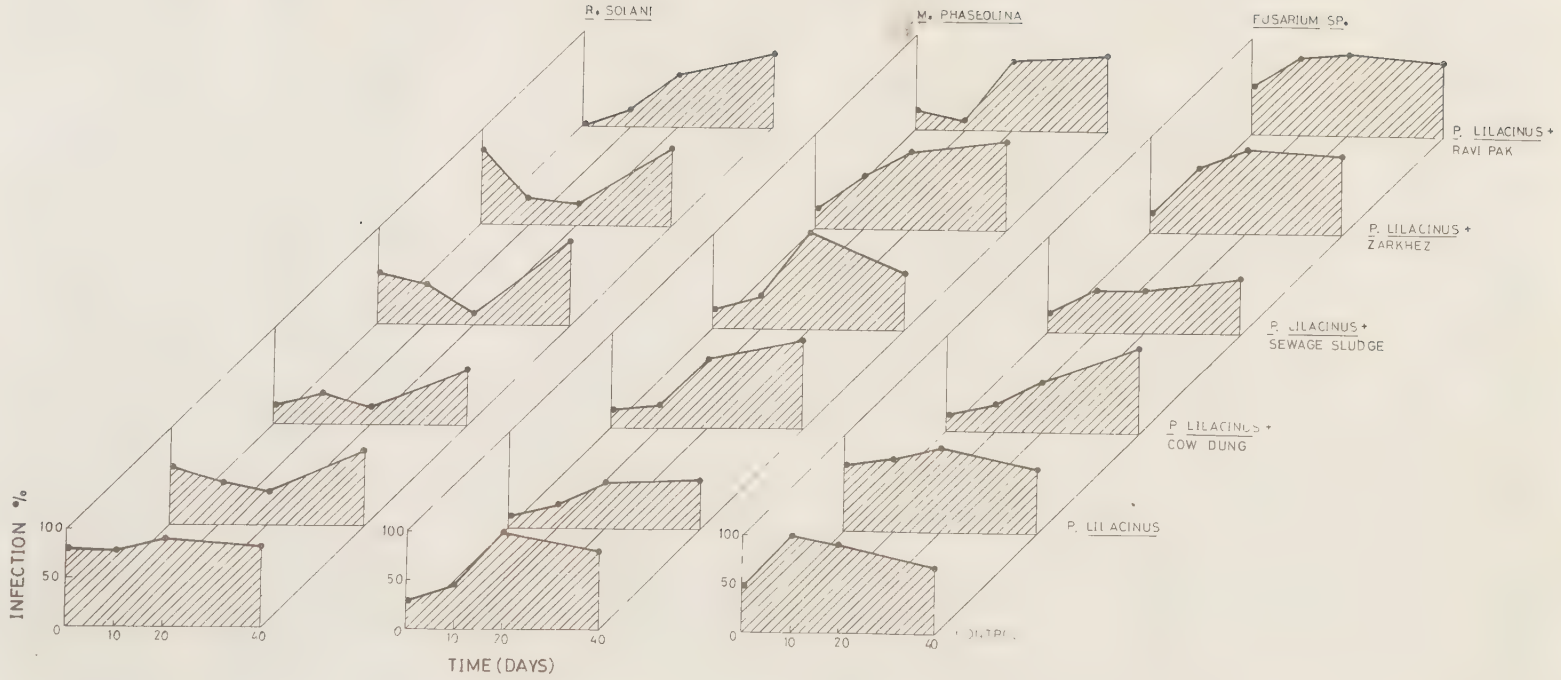

Fig. 26. Effect of different organic fertilizers on the efficacy of Paecilomyces lilacinus against soilborne root infecting fungi.

after 7, 15, 30 and 60 days interval and population of M. phaseolina was assessed by wet sieving and dilution method (Sheikh & Ghaffar, 1975).

In non-amended soil the population of M. phaseolina sclerotia showed an increase of 30% over the initial population after 60 days sampling. Wheat straw used either @ 1 or 10% w/w, or molasses used @ 1% w/w showed no reduction in M. phaseolina populations but populations increased respectively by upto 37 and 27% over the initial populations after 60 days of amendment. In treatments where molasses was used @ 10% w/w 11% reduction in M. phaseolina population was observed after 15 days but population again increased and after 60 days of treatment M. phaseolina population was 2% greater than the initial population. It is interesting to note that lucerne used @ 1 or 10% w/w, respectively, showed a reduction of 18 and 87% after 15 days of amendment whereas after 30 days of amendment M. phaseolina population in 1 and 10% amendment series was 14 and 24% greater than the initial populations. After 60 days of amendment the population of sclerotia again declined to the initial level in lucerne amendment used @ 10% w/w (Fig. 27). Such similar observations have been made by Ghaffar and Erwin (1969).

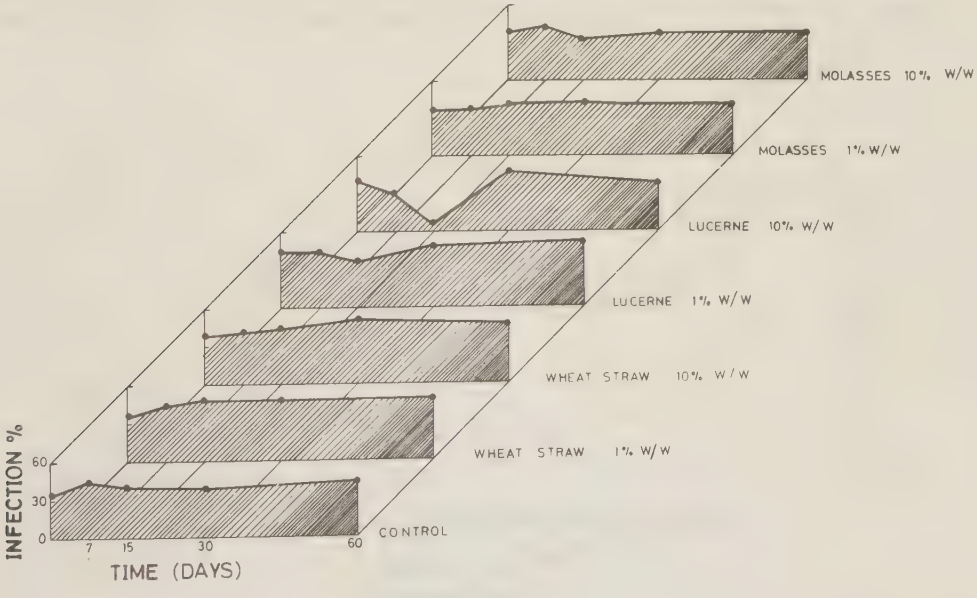

Fig. 27. Effect of organic amendment on the population of Macrophomina phaseolina in soil.

Expt.ii. Soil artificially infested with R. solani 1% w/w was amended with root and shoot fragments of moth (Vigna aconitifolia (Jacq.) Marechal) @ 1 and 5% w/w. Non-amended soil served as control. Soil kept in nylon bags was buried in field and removed to assess R. solani population at 0-time and after 2, 4, 8 and 12 week interval. Population of R. solani was assessed by baiting technique (Wilhelm, 1956). Using sterilized millet seeds as bait the percentage of colonized seeds was recorded to determine the population of R. solani in soil.

Population of R. solani in non-amended soil showed gradual decline upto 8 weeks (16%) but after 12 week interval R. solani population increased by upto 10% as compared to initial population. In soil amended with moth @ 1% w/w R. solani population showed a reduction of 42% upto 8 weeks but after 12 weeks of treatment R. solani population increased by upto 23% as compared to the population of 8 week interval. Soil amended with moth @ 5% w/w showed a gradual reduction in R. solani population and after 12 weeks 36% reduction was observed over the initial population (Fig. 28).

Expt.iii. Soil of experimental plots at the Department of Botany, University of Karachi, was amended with root and shoot pieces of moth @ 1% w/w. Non-amended soil served as control. Seeds of mung bean were sown and infection of roots by root infecting fungi was assessed after 2, 4, 8 and 12 week interval.

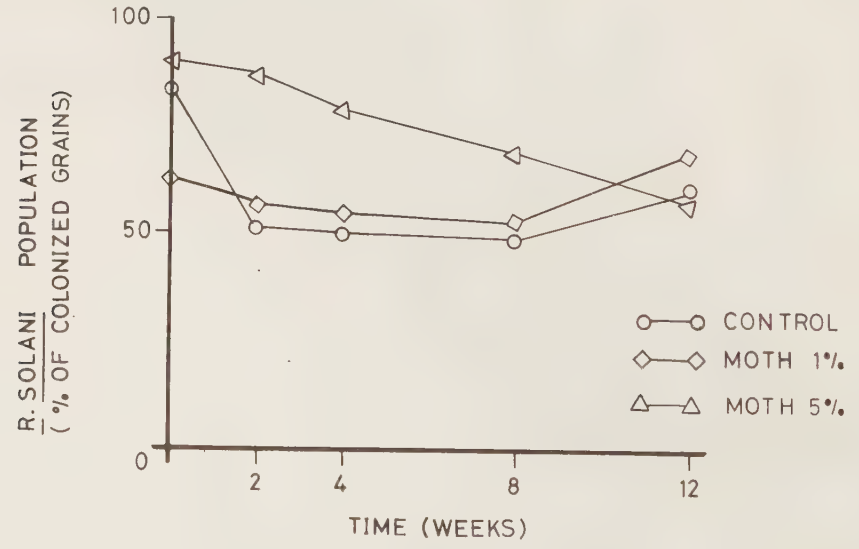

Fig. 28. Effect of soil amendment with moth on population of Rhizoctonia solani in soil.

After 2 and 4 weeks infection of roots by R. solani reduced by 51% in amended soil as compared to control but after 12 weeks 100% infection was observed both in amended and natural soil. Infection of roots by M. phaseolina in amended soil was 25% less than that in non-amended soil after 2 weeks but after 4 weeks there was no significant difference in infection and after 8 and 12 weeks infection was 100% both in amended and nonamended soils. No significant difference in Fusarium infection was observed in amended and non-amended soils (Fig. 29).

g. Combined effect of microbial antagonists and organic substrate

Wheat bran cultures of microbial antagonists were found more effective than conidial preparations in reducing population of R. solani and the incidence of damping-off of radish, cotton and sugar beet (Lewis & Papavizas, 1985). It suggested that hyphae already occupying the food base are not affected by fungistasis and the activity of mycelial preparation in enhacement of antagonists density coupled with suppression of pathogen prevented damping-off. Effect of the combined use of microbial antagonists and different organic substrates on the population of M. phaseolina was, therefore, evaluated.

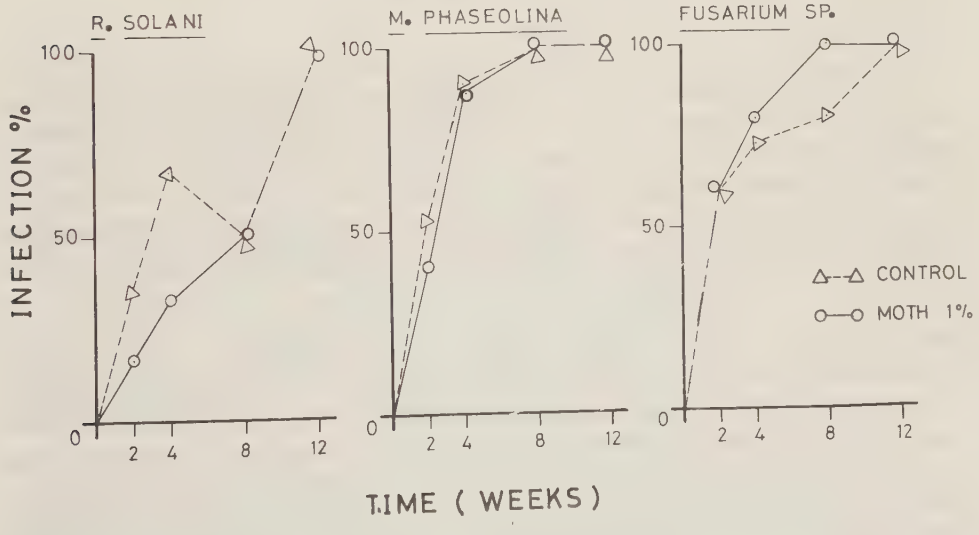

Fig. 29. Effect of soil amendment with moth on infection of mung bean roots by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp.

Expt.i. Soil artificially infested with M. phaseolina @ 30 scl./g of soil was amended with organic substrates viz., wheat straw, lucerne or molasses @ 1 and 10% w/w. Soil was inoculated separately with conidial suspensions of microbial antagonists viz., A. candidus, C. cladosporioidis and T. roseum. Microbial antagonists were also used after growing on organic substrates for 48 h prior to their application to soil. Soil samples were removed at 0-time and after 7, 15, 30 and 60 days of treatment to assess the population of M. phaseolina in soil.

In control soil or where soil was amended with conidial suspension of microbial antagonists the population of M. phaseolina after 7, 15, 30 and 60 days was greater than initial population. Similarly when wheat straw was used alone or alongwith microbial antagonists either @ 1 or 10% w/w, no reduction in M. phaseolina population was observed. Lucerne when used @ 10% w/w either alone or with A. candidus, C. cladosporioidis or T. roseum, the population of M. phaseolina after 15 days of treatment reduced respectively by upto 87, 94, 97 and 97% as compared to the initial populations. After 60 days the population of M. phaseolina increased by upto 0% (control), 11% (A. candidus), 52% (C. cladosporioidis) and 39% (T. roseum) than the initial populations. Lucerne @ 1% w/w and molasses @ 1 and 10% w/w used either alone or mixed with microbial antagonists showed no significant reduction in M. phaseolina population (Fig. 30).

h. Effect of soil solarization

Soil solarization is an effective means for controlling soilborne pathogens by reducing their populations (Katan, 1985). Populations of sclerotia of M. phaseolina (Sheikh & Ghaffar, 1984) and S. oryzae (Usmani & Ghaffar, 1982) have been eliminated by soil solarization. Efficacy of soil solarization for the control of R. solani, M. phaseolina and Fusarium spp., was, therefore, examined.

Expt.i. In July, 1987, nylon bags containing 300 g soil, artificially infested with soil-wheat meal culture of R. solani (@ 1% w/w) were buried in field at 0-5 and 15-20 cm depths and covered with transparent plastic sheets. The soil was brought to field capacity before mulching. Nylon bags in non-mulched plots served as control. Bags were removed at 0-time and after 7 and 15 days of mulching treatment. Maximum soil temperature in mulched plots was 48°C as compared to 39°C in non-mulched plots. Soil was transferred to 8 cm

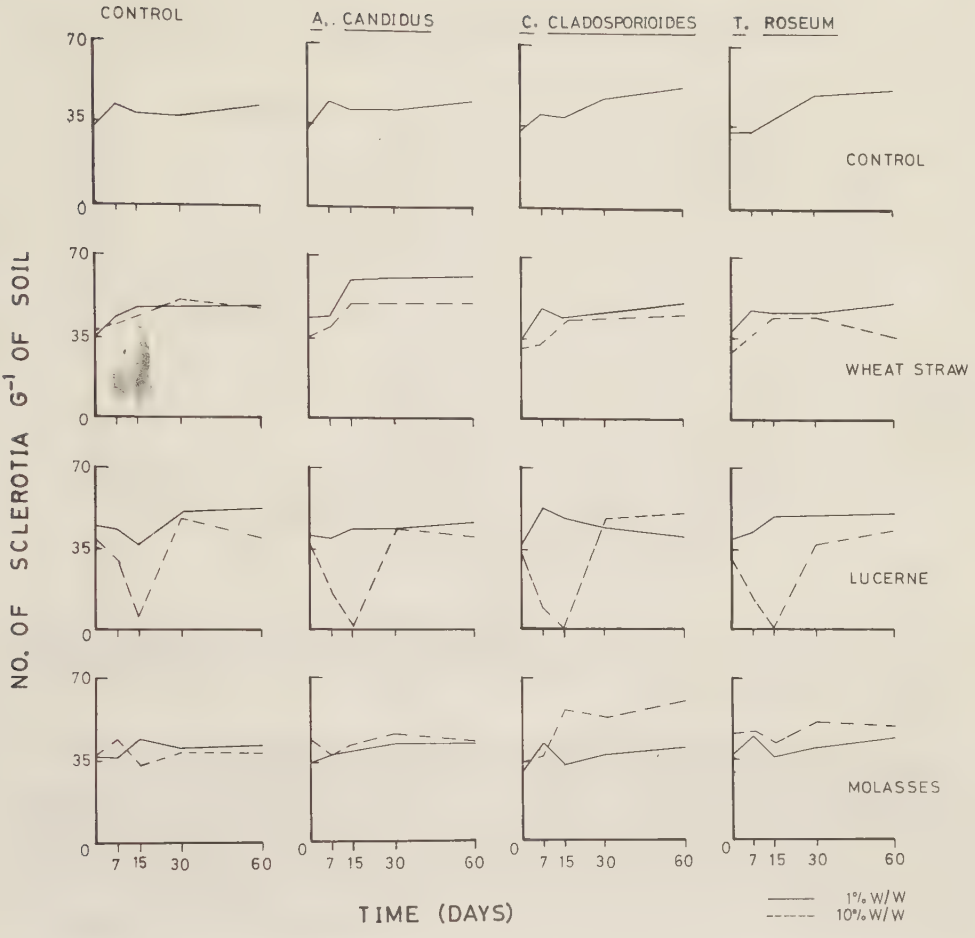

Fig. 30. Combined effect of microbial antagonists and organic amendment on population of Macrophomina phaseolina in soil.

diameter plastic pots, 250 g in each, and 10 seeds of mung bean planted in each pot to see R. solani infection. Populations of R. solani in mulched and non-mulched soils were also determined by baiting tachnique, using sterilized millet grains as a bait. Percentage of grains colonized was recorded as the population of R. solani in soil.

Population of R. solani in non-mulched soil showed no significant change both at 0-5 and 15-20 cm depths even after 15 days whereas in mulched soil population of R. solani reduced to zero at 0-5 cm depth after 7 days and at 15-20 cm depth after 15 days of mulching treatment (Fig. 31).

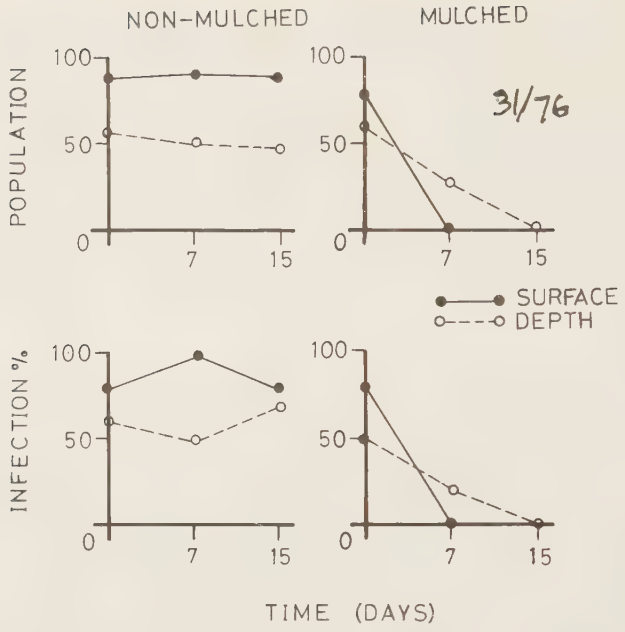

Fig. 31. Effect of plastic mulching treatment on the population of Rhizoctonia solani and its infection on mung.

No R. solani infection of mung bean was observed in mulched soil removed from 0-5 cm depth after 7 days or from 15-20 cm depth after 15 days of mulching treatment. Infection of roots by R. solani showed no significant difference in non-mulched soil since at 0-5 cm depth R. solani infection was 89% at 0-time and 90% after 15 days interval (Fig. 31).

Expt.ii. Experimental plots at the Department of Botany, University of Karachi were mulched for 7 days using transparent polyethylene sheets during July, 1987. Polyethylene sheets were removed after 7 days. The soil was then turned over and remulched for another 7 days period. Non-mulched soil served as control. Seeds of mung bean were sown and root samples were collected after 5, 10, 15 and 30 days to assess the colonization of roots by soilborne root infecting fungi.

Infection of root by R. solani on 5 days old plants was 0% both in mulched and non-mulched treatments. After 5 days, R. solani infection gradually increased in non-mulched plots as compared to mulched plots. After 30 days 45% plants in mulched soil were infected by R. solani as compared to 60% in nonmulched treatment. Similarly infection of roots by M. phaseolina and Fusarium spp., in mulched soil after 30 days was respectively 56 and 21% less than that in non-mulched soil (Fig. 32).

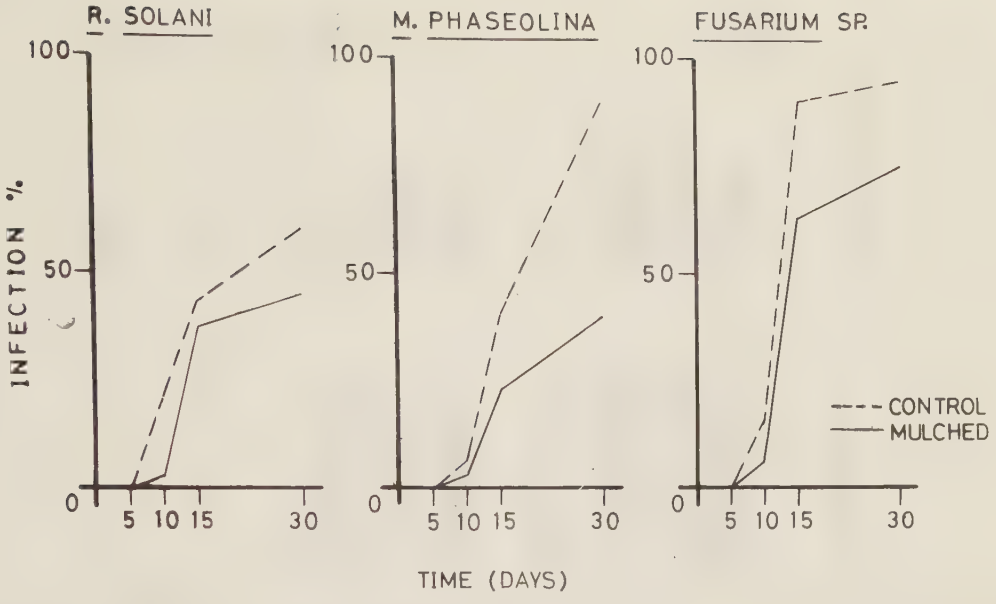

Fig. 32. Effect of plastic mulching treatment on infection of mung roots by Rhizoctonia solani, Macrophomina phaseolina and Fusarium sp.

Soil solarization delayed the development of disease. Integrated use of plastic mulching with microbial antagonists and or organic amendment may provide better protection to plants.

i. Effect of soil moisture

Soil moisture is reported to have varied effects upon disease development. Water stress conditions are known to increase severity of root rot of sorghum (Hsi, 1961; Edmunds, 1964) and cotton (Ghaffar & Erwin, 1969). Similarly F. oxysporum f.sp. pisi the cause of pea wilt, developed more rapidly in soil at 90% than at 40% MHC (Linford, 1928). High soil moisture generally decays the fibrous root system, besides a direct effect of a reduced oxygen supply upon subterranean organs (Walker, 1969). Experiments were therefore carried out to study the effect of soil moisture on infection of F. oxysporum f.sp. lycopersici on tomato and M. phaseolina on sunflower.

Expt.i. Tomato seeds were planted in soil naturally and artificially infested with F. oxysporum f.sp. lycopersici and the soil was maintained at 25, 50, 75 and 100% moisture level. Rate of seed germination and incidence of Fusarium

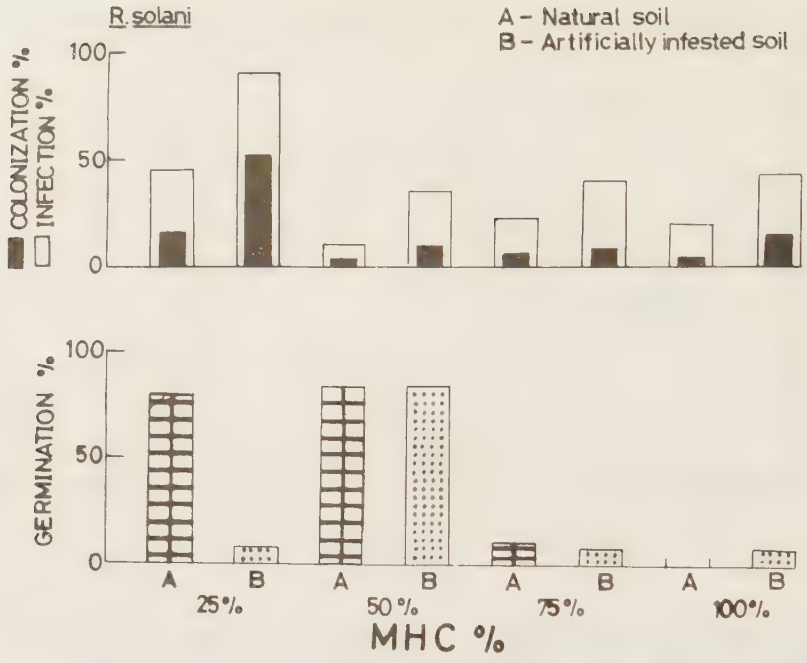

Fig. 33. Effect of soil moisture on infection of tomato roots by Fusarium oxysporum f.sp. lycopersici.

infection on tomato was low at 75 and 100% MHC. Maximum seed germination with low Fusarium infection was observed where soil was kept at 50% MHC whereas at 25% MHC the rate of seed germination was low with severe seedling infection by F. oxysporum (Fig. 33). Predisposition of plants to infection under water stress was observed and there was a correlation of the concentration of inoculum with infection.

Expt.ii. Seven day old sunflower seedlings were transplanted into 15 cm diameter plastic pots containing soil artificially infested with M. phaseolina @ 25 scl./g of soil. Soil moisture was adjusted and maintained at 25, 50, 75 and 100% MHC. A comparable set with non-infested soil was used as control. Plants did not survive at 100% MHC both in infested and non-infested soil. After 30 days growth infection of roots by M. phaseolina in control and artificially infested soil was 86 and 100% at 25% MHC, 78 and 100% at 50% MHC and 71 and 100% at 75% MHC respectively (Fig. 34). Mortality of plants both in control and artificially infested soil was highest at 50% MHC (14 and 23%) than at 25% MHC (9 and 11%) and 75% MHC (11 and 17%) indicating that M. phaseolina infection in natural soil decreased with increase in soil moisture but very high soil moisture (100% MHC) was found lethal for plants.

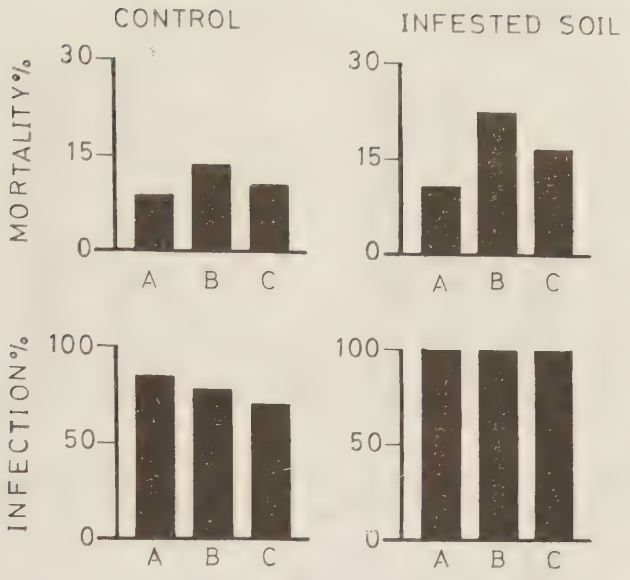

Fig. 34. Effect of soil moisture on Macrophomina phaseolina infection of sunflower.

A= 25% MHC B= 50% MHC C= 75% MHC

Expt.iii. Seven day old sunflower seedlings were transplanted to 15 cm diameter plastic pots containing soil with a natural infestation of 5 scl./g of soil or artificially infested with M. phaseolina (30 scl./g of soil). Soil moisture was maintained at 75% MHC. After 30 days of transplantation, plants were subjected to water stress till the soil moisture reduced to 10% MHC (on the basis of daily weight). Plants were maintained at 10% MHC for 3 days after which 75% MHC was regained. Only 15 and 14% plants recovered in control and infested soil respectively, while 85 and 86% plants wilted in control and infested soil rspectively (Fig. 35). M. phaseolina was isolated from roots of all infected plants indicating that water stress conditions predisposed plants to root infection by M. phaseolina.

Expt.iv. In another experiment plants were grown in soil naturally or artificially infested with M. phaseolina. One set of plants both in naturally (10 scl./g of soil) and artificially infested soil (41 scl./g of soil) was regularly watered while one comparable set (25 and 63 scl./g of soil) was watered after 7-10 days interval. After 30 days root infection by M. phaseolina in regularly watered plants was 60% with 16% colonization of root fragments in natural soil and 100% with

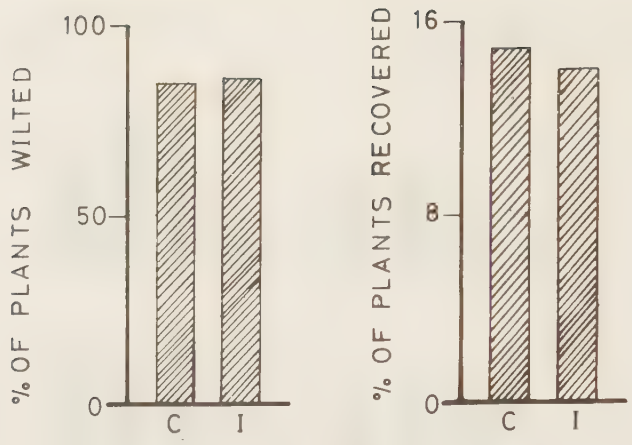

Fig. 35. Effect of water stress on Macrophomina phaseolina infection of sunflower. C= Control I= Infested soil

72% colonization in artificially infested soil. In water stressed plants root infection was 100% both in naturally and artificially infested soil with 60 and 85% colonization of root fragments respectively (Fig. 36). Water stress would appear to predispose plants to greater colonization by M. phaseolina as also reported for cotton (Ghaffar & Erwin, 1969) and sorghum (Edmunds, 1964).

j. Root rot and root knot disease complex

In our field studies infection by soilborne root infecting fungi was frequently found associated with infection by root knot nematodes. Use of nematicides is generally recommended against plant parasitic nematodes but keeping in view the cost of chemicals and hazards involved, biological control has been suggested as an alternative method of control. Dr Parvez Jatala of the International Potato Centre (CIP), Lima, Peru, discovered a soilborne fungus Paecilomyces lilacinus which parasitises the eggs of Meloidogyne incognita and destroys the embryo within 5 days. P. lilacinus is known to control the population of root knot nematode both under field and green house conditions consistently and efficiently. In our experiments P. lilacinus was found to inhibit the growth of M. phaseolina and R. solani in agar plate cultures. Since nematodes are known to predispose plants to fungal infection, efficiency of P. lilacinus against M. incognita, R. solani and M. phaseolina on mung (Vigna radiata (L.) Wilczek) and okra (Abelmoschus esculentus (L.) Moench) under field condition was tested during June 1986. For comparison the effect of Furadan, a generally recommended nematicide was

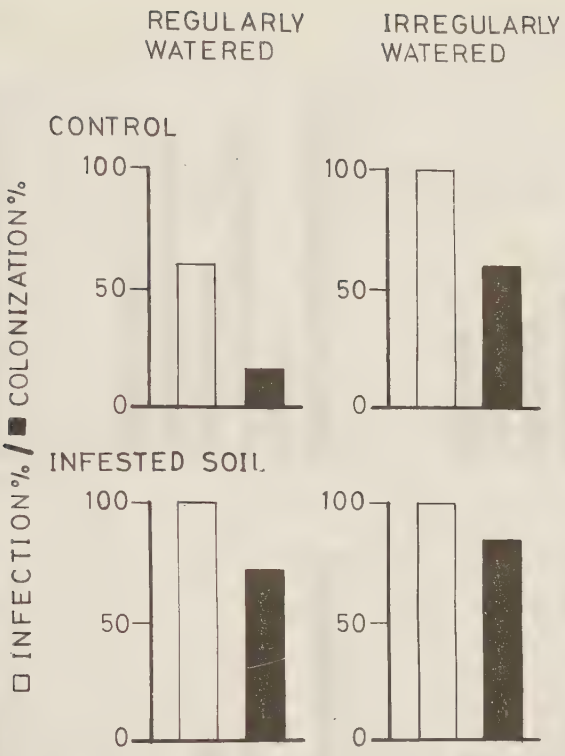

Fig. 36. Effect of water stress on infection and colonization of sunflower roots by Macrophomina phaseolina.

examined. P. lilacinus grown on rice grains was applied in furrows @ 40 g/m whereas Furadan was used @ 13 and 26 Kg/acre alone or in combination with P. lilacinus.

It is interesting to note that infection of M. incognita and R. solani decreased significantly in all the treatments. Better results were obtained where P. lilacinus was used alone. Similarly M. phaseolina infection reduced respectively, by 33 and 45% on mung and okra only where P. lilacinus was used alone. Furadan used alone or in combination with P. lilacinus was found less effective in controlling root-rot and root-knot disease on mung and okra (Fig. 37).

In another experiment the effect of P. lilacinus as a biocontrol agent was compared with pesticides viz., Furadan (Nematicide), Agrosan and Vitavax (Fungicides) on the control of root rot - root knot disease of tomato during August, 1986. These were either applied in soil or the tomato seedlings were dipped in a solution of pesticides or P. lilacinus. P. lilacinus when used as soil treatment reduced root knot index (RKI) to zero and the infection by R. solani

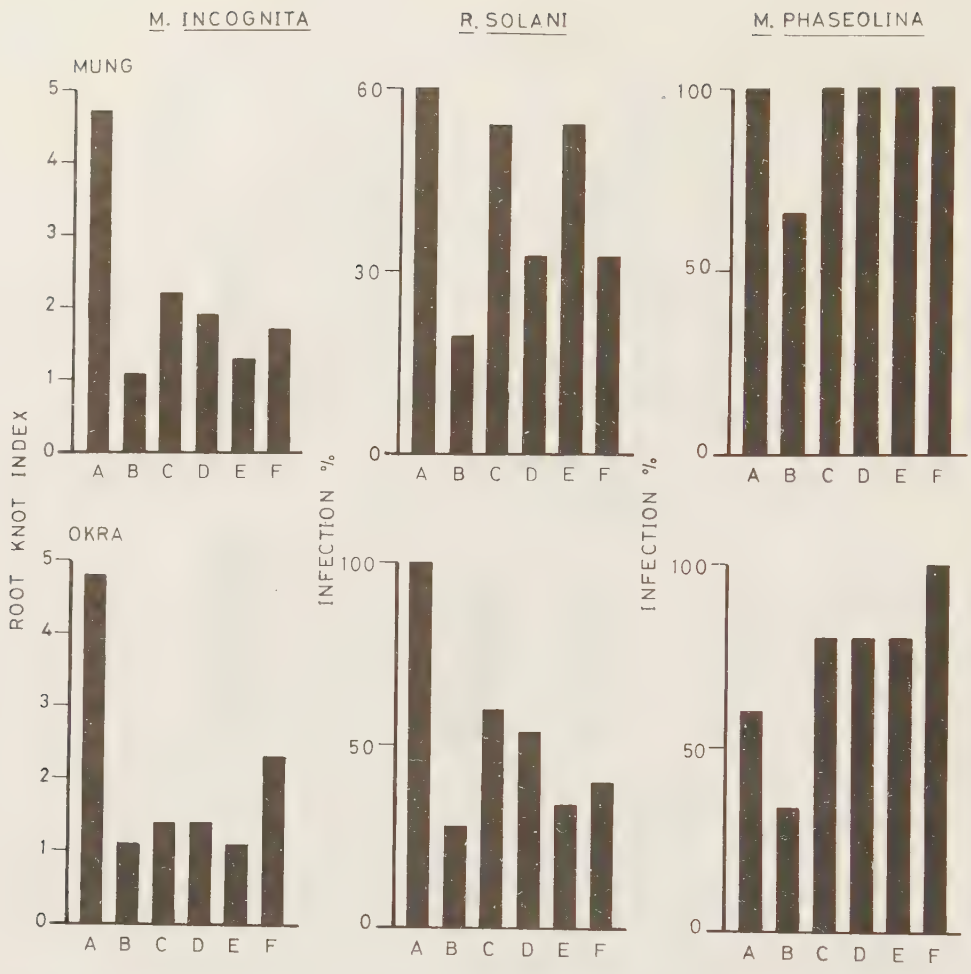

Fig. 37. Effect of soil treatment with Paecilomyces lilacinus and Furadan on root rot - root knot disease complex of mung and okra.

A= Control **B**= P. lilacinus **C**= Furadan @ 13 Kg/acre **D**= Furadan @ 26 Kg/acre **E**= P. lilacinus+ Furadan @ 13 Kg/acre **F**= P. lilacinus+ Furadan @ 26 Kg/acre.

was reduced by 80%. Agrosan, when used as seedling treatment, reduced R. solani infection to zero and RKI from 5 to 2 (Fig. 38). Infection by M. phaseolina was reduced by upto 20% where Agrosan was drenched in soil.

During August, 1986, a similar experiment was carried out at the farmers field in Korangi area, Karachi where soil naturally infested with M. javanica, R. solani and Fusarium

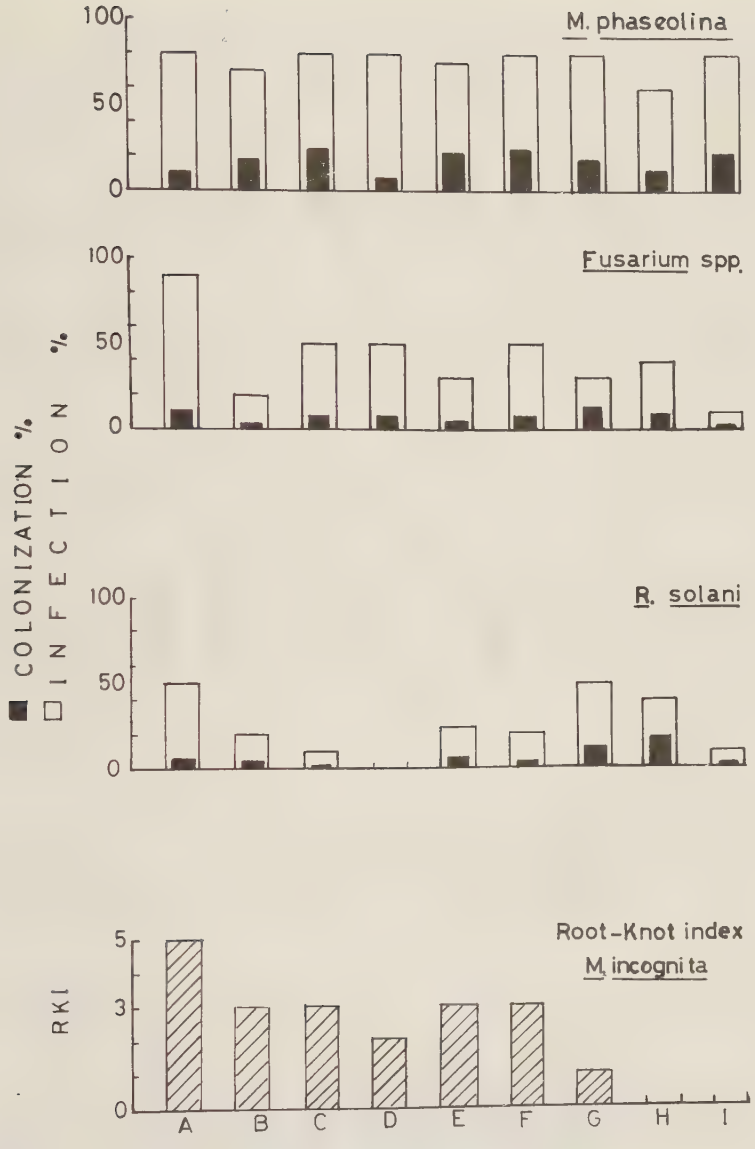

Fig. 38. Effect of Paecilomyces lilacinus, Furadan, Vitavax and Agrosan on the root rot - root knot disease complex of tomato.

A= Control **B**= Seedlings dipped in P. lilacinus suspension **C**= Seedlings dipped in Vitavax solution **D**= Seedlings dipped in Agrosan solution **E**= Furadan mixed in soil **F**= Conidial suspension of P. lilacinus drenched in soil **G**= Vitavax solution drenched in soil **H**= Agrosan solution drenched in soil **I**= P. lilacinus culture grown on rice grains mixed in soil.

spp., was used. P. lilacinus was more effective against M. incognita than M. javanica on tomato. RKI reduced from 5 to 3 in all treatments except where Agrosan or Vitavax was drenched in soil or seedlings were dipped in Vitavax solution (Fig. 39). Infection of roots by R. solani reduced by 100% in all the treatments except where Agrosan was drenched in soil or P. lilacinus, multiplied on wheat straw was used. The infection of Fusarium spp., reduced by 100% where seedlings were dipped in solution of Vitavax or Furadan was mixed in soil. No other treatment reduced Fusarium infection on tomato over control (Fig. 39). Infection of roots by M. phaseolina was not observed in any of the treatments.

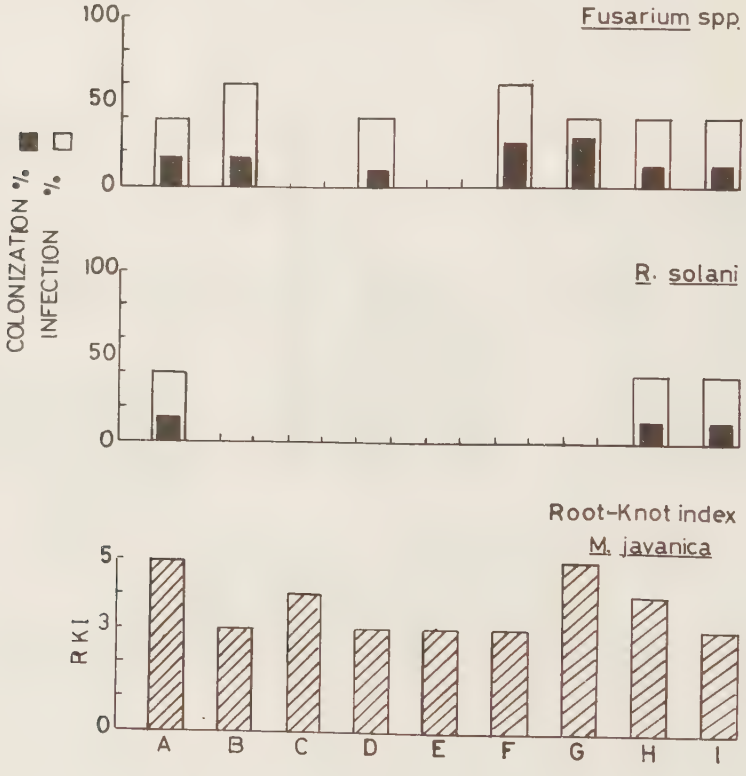

Fig. 39. Effect of Paecilomyces lilacinus, Furadan, Vitavax and Agrosan on root rot - root knot disease complex of tomato.

A= Control B= Seedlings dipped in P. lilacinus suspension C= Seedlings dipped in Vitavax solution D= Seedlings dipped in Agrosan solution E= Furadan mixed in soil F= Conidial suspension of P. lilacinus drenched in soil G= Vitavax solution drenched in soil H= Agrosan solution drenched in soil I= P. lilacinus culture grown on wheat straw, mixed in soil.

Another experiment was carried out at Haji Murad Jaffer Bagh, Malir, Karachi, where the soil naturally infested with M. javanica, R. solani, M. phaseolina and Fusarium sp., was used. P. lilacinus was used either as seed dressing or amended to soil after 24 hours growth on wheat straw. Efficacy of P. lilacinus was compared with Furadan (used @ 16 Kg granules/acre) and Mocap (used @ 16 and 32 Kg granules/acre). Sugarbeet (Beta vulgaris L.) was used as test plant.

RKI reduced in all the treatments but Mocap was more effective than P. lilacinus and Furadan. There was no significant difference in RKI in P. lilacinus and Furadan treated plants (Fig. 40). Infection of sugarbeet roots by R. solani reduced respectively by 72 and 30% where P. lilacinus was used as seed dressing or Mocap @ 16 Kg granules/acre was used. Similarly a 15 and 7% reduction in Fusarium infection was observed where P. lilacinus was used as seed dressing or Mocap was applied to the field (Fig. 40). Root infection by M. phaseolina reduced by upto 60% in Mocap (used @ 32 Kg granules/acre) and P. lilacinus were amended to soil. Where Furadan was used no significant reduction in M. phaseolina infection was observed.

It is interesting to note that P. lilacinus was more effective against M. incognita than M. javanica. A judicious use of a pesticide and or biocontrol agent could thus be used.

K. Persistence of the effect of P. lilacinus and Furadan

In our experiments on the control of root rot - root knot disease complex, P. lilacinus was found more effective than Furadan used either @ 13 or 26 Kg granules/acre alone or combined with P. lilacinus. Persistence of the effects of P. lilacinus and Furadan was tested on gram (Cicer arietinum L.) during the next cropping season. Seeds were sown in the same field during December, 1986, without any additional amendment of P. lilacinus and Furadan and after 60 days growth RKI and infection of roots by R. solani and M. phaseolina was assessed. It is interesting to note that P. lilacinus showed a residual effect and reduced root knot infection (RKI from 4.7 to 2) whereas infection of roots by M. phaseolina and R. solani respectively reduced by upto 40 and 50% on gram. Furadan did not give significant residual effects over the control (Fig. 41).

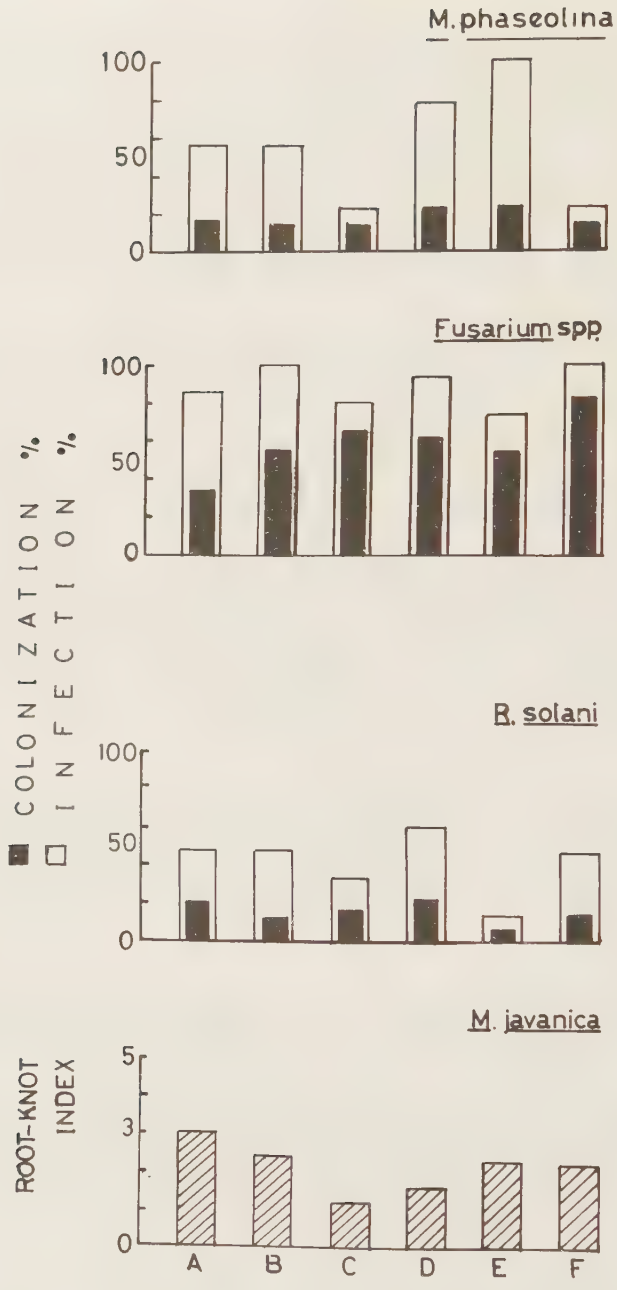

Fig. 40. Effect of Paecilomyces lilacinus, Furadan and Mocap on root rot - root knot disease complex of sugarbeet.

A= Control B= Furadan @ 16 Kg/acre C= Mocap @ 32 Kg/acre D= Mocap @ 16 Kg/acre E= Seed dressing with P. lilacinus F= P. lilacinus grown on rice grains.

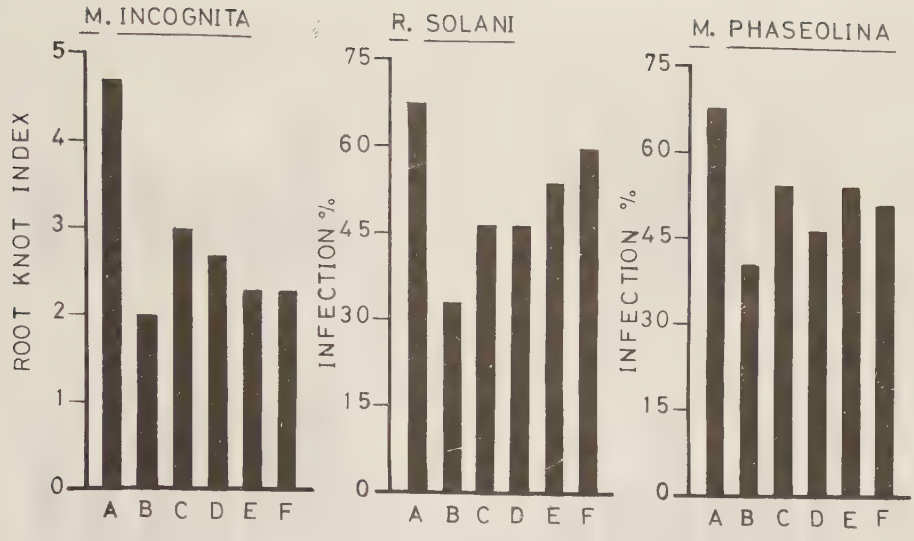

Fig. 41. Persistence of the effect of Paecilomyces lilacinus and Furadan in the control of root rot - root knot disease complex on gram.

A= Control **B**= P. lilacinus **C**= Furadan @ 13 Kg/acre **D**= Furadan @ 26 Kg/acre **E**= P. lilacinus+ Furadan @ 13 Kg/acre **F**= P. lilacinus+ Furadan @ 26 Kg/acre.

1. Delivery of P. lilacinus in soil

In view of promising results on the efficacy of P. lilacinus in the control of root rot - root knot disease complex, experiments were carried out to study the effective means for the delivery of P. lilacinus in soil.

P. lilacinus was used either as seed dressing or incorporated into soil in furrows @ 50 g/m after growing on wheat straw, rice straw, rice grains and sorghum grains for 3 weeks at room temperature. Seeds of mung bean were sown in February, 1987, and after 60 days growth plants were uprooted for the assessment of RKI and R. solani and M. phaseolina infection.

Germination of seeds increased where P. lilacinus culture, multiplied on rice grains was used (21%) as compared to rice straw (19%) or used as seed dressing (8%). Where wheat straw or sorghum grains were used as a food base, germination of seeds however decreased by 29 and 18% respectively (Fig. 42).

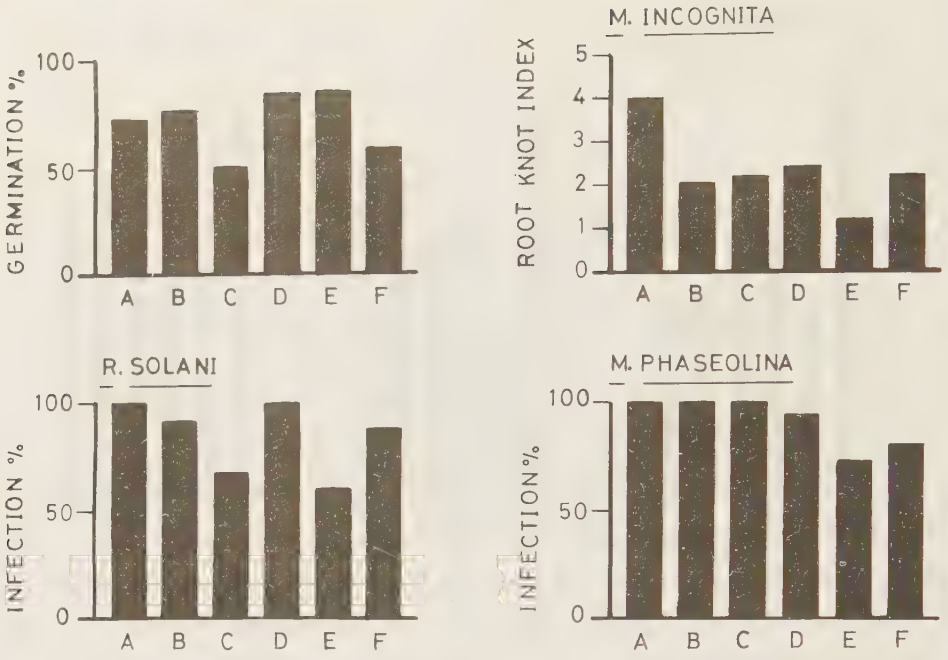

Fig. 42. Effect of seed dressing and different substrates for growth of P. lilacinus and its field application for the control of root rot - root knot disease complex on mung.

A= Control B= Seed dressing C= Wheat straw D= Rice straw E= Rice grains F= Sorghum grains.

Use of rice grains as a food base was found most effective in the control of root knot and root rot infection, RKI reduced to 1.2 as compared to 4 in control whereas 40% reduction in R. solani and 27% in M. phaseolina infection was observed over the control. Seed dressing or use of rice straw, wheat straw and sorghum grains were less effective than rice grains where RKI was 1.9, 2.4, 2.2 and 2.2 and the reduction in infection of roots by R. solani was 8, 0, 33 and 13% respectively whereas M. phaseolina infection was respectively reduced by upto 0,7,0 and 20% (Fig. 42).

IV. Diagnostic and Advisory Service

Root rot specimens of betel vine, chikoo, chillies, coconut, guava, holy basil, jack fruit, mango, melon, mulberry, okra, pea, papaya, sugarbeet, tomato and water melon received from the farmer's fields at Malir, Korangi, Landhi, Dumlotee, Gadap, Gharo, Thatta, Badeen and Thur areas were examined and the disease causing organisms isolated and identified. Methods for the control of root rot infection

were recommended. Coconut plants sprayed with a mixture of Benomyl and Malathion showed pronounced recovery from root and stem rot caused by R. solani and Botryodiplodia palmarum. Vitavax used as soil drench was also found effective. Similarly, soil treatment with conidial suspension of Paecilomyces lilacinus before transplanting tomato seedlings was suggested against root rot - root knot disease complex in Malir. Treated plants showed better growth and less root knot - root rot infection caused by Meloidogyne javanica, R. solani and M. phaseolina. Dithane was found to provide better protection to the betel vines from root rot, whereas jack fruit at Landhi suffering from Botryodiplodia root rot showed pronounced recovery where Captan was drenched in the field.

A total number of 64 cultures of fungi, received from Mr. IkramulHaq, Lecturer, Government College Lahore, 12 cultures from Mr. Abdul Rahim Magsee, Research Assistant, Cotton Research Institute, Sakrand, 8 from Horticultural Institute Mirpurkhas and 12 cultures from Mrs. Hajra Azhar Ali, Assistant Professor of Plant Physiology Section, University of Karachi were identified.

V. Training Course on DIAGNOSIS AND CONTROL OF SOILBORNE PLANT DISEASES

A training course on the 'Diagnosis and Control of Soilborne Plant Disease' was organized from 1-10 December, 1987 at the Department of Botany, University of Karachi. Atleast 40 candidates belonging to different research and or educational institutions of Pakistan viz., Agriculture Research Institute (ARI), Tandojam, ARI Sariab, ARI Tarnab, Peshawar; Atomic Energy Agriculture Research Institute, Tandojam; Pakistan Tobacco Board, Peshawar; NIAB Faisalabad, CDRI/NARC, Islamabad; CDRI, Karachi; Department of Plant Protection Karachi, PCSIR Laboratories, Karachi; NWFP Agriculture University, Peshawar; Federal. Govt. Urdu Science College, Karachi; Sir Syed Girls College, Karachi; Department of Genetics, University of Karachi; Department of Botany, University of Karachi; Jaffer Brothers (Pvt) Karachi and Horticulturists and Progressive Farmers of Karachi and its suburbs, participated in the training course. During the course, lectures and practical works dealing with rapid diagnostic procedures for soilborne diseases were conducted. The symptoms of diseases, techniques of isolation, description of pathogens leading to the identification of some soilborne disease causing organisms viz., Rhizoctonia

solani, Macrophomina phaseolina, Fusarium spp., Botryodiplodia theobromae, Sclerotinia sclerotiorum, Pythium spp., Phytophthora spp., Verticillium spp., Drechslera spp., Sclerotium rolfsii, S. oryzae and Streptomyces scabies, artificial disease development and methods of control under laboratory and field conditions were discussed. Lecture notes and brochures related to the topics were also distributed. A field trip was arranged for the trainees where incidence of root rot diseases under natural field conditions was observed, diseased specimens were collected to study the disease causing organisms. Certificates were awarded to the participants after successful completion of the course.

The course was organized under the MART program. We are grateful to Dr. Amir Muhammad, Chairman, PARC for his inaugural address, to Dr. Manzooruddin Ahmed, Vice-Chancellor, for his presidential address, to Dr. S.I. Ali for his concluding remarks. The certificates after successful completion of the training course were awarded to the trainees by Dr. Abdul Kafi, the Director and Plant Protection Adviser, Government of Pakistan, Karachi. Dr. A. Ghaffar, Dr. M. Jalaluddin, Mr. M.H. Hashmi and members of the staff of SDRC helped us conduct the training program.

Discussion

Whereas comprehensive lists of fungi attacking above ground portions of plants have been reported (Mirza & Qureshi, 1978; Ghaffar & Kafi, 1968; Ghaffar et al,1971; Ghaffar & Abbas, 1972), fungi inhabiting soil and attacking underground portions of plants have received little attention. The present report gives an account of 91 plant species examined from which 52 new hosts of R. solani, 44 of M. phaseolina, 2 of S. rolfsii, 6 of B. theobromae, 3 of Melanospora sp., 44 of D. australiensis and 12 of D. halodes have been encountered. With use of selective isolation techniques the presence of other soilborne root infecting fungi like the pythiaceous fungi and species of Verticillium etc., could be detected. There is thus a need that comprehensive study of soilborne root infecting fungi should be continued. Damages of 10-80% by soilborne root infection on vegetable crops and fruit trees have been noticed. There is need to evaluate this study over a period of time on different crop plants.

In our in vitro experiments fungicides viz., Agrosan, Benomyl, Captan, Dithane, Fernasan and Vitavax when used

@ 1,000 and 10,000 ppm inhibited growth of M. phaseolina, R. solani, Fusarium sp., B. theobromae and S. rolfsii but application of fungicides at such high doses would be uneconomical. Windels et al (1985) considered the use of fungicides for seed treatment as the most economical method for disease prevention. Treatment of seeds with Vitavax or Benomyl effectively reduced R. solani infection on sugarbeet (Ahmadinejad, 1973) and cotton (Al-Beldawi & Welleed, 1973) whereas Benomyl prevented M. phaseolina infection on cotton (Ilieseu et al, 1985). This is contrary to our results where fungicides like Benomyl and Vitavax failed to produce significant reduction in M. phaseolina and R. solani infection on mung and okra indicating that eradication of fungal resting structures may be difficult with acceptable and reasonable rate of fungicidal application as also reported by Papavizas (1977). Soil treatment with fungicides reduced R. solani infection on sunflower in Benomyl but not in Captan treatment, and M. phaseolina infection in Captan but not Benomyl treatment indicating selective action of fungicides. A combined use of Benomyl and Captan reduced infection of roots by M. phaseolina, R. solani and Fusarium sp. Similar results have been reported by Seoud et al (1982) where combined use of Vitavex, Captan and Deconial 2781 was found most effective against F. oxysporum, R. solani and M. phaseolina on sesame.

Since all herbicides sooner or later reach the soil, irrespective of the mode of application, the soil microflora is most effected by them. The effect may be stimulatory or inhibitory to a specific pathogen (Katan & Eshell, 1973; Altman & Campbell, 1977). In our pot experiment, soil treatment with Basfapon @ 100 ppm resulted in 20% reduction in R. solani infection on okra whereas Saturn was ineffective against R. solani. There seems to be a significant interaction between the herbicides and the soilborne root infecting fungus R. solani and or soil micro-organisms.

Seeds are vulnerable to seedborne or soilborne pathogens (Kommedahl & Windels, 1981). Microbial antagonists may therefore be more effective when applied to seed than when applied to soil because of their proximity to the infection court. Lewis and Papavizas (1985) found that actively growing hyphae of microbial antagonists in bran culture were more effective against R. solani. In our experiments use of microbial antagonists viz., Arachniotus sp., A. candidus, G. virens, P. lilacinus, T. harzianum, T. hamatum, T. flavus, Streptomyces sp., B. subtilis, R. leguminosarum and R. meliloti, after multiplication on wheat straw gave better results

against M. phaseolina infection on mung and okra than their use as seed dressing whereas on cotton, use of microbial antagonists as seed dressing was more effective than soil application. However soil treatment with microbial antagonist after their multiplication on wheat straw was less effective than seed dressing against R. solani infection. Use of rice grains as a substrate for microbial antagonists showed better protection against M. phaseolina, R. solani and Fusarium infection on okra. Perhaps it would be a matter of technology as well as ecology to find ways to make biocontrol effective and competitive with chemical seed or soil treatment.

Cultures of microbial antagonists previously grown on wheat straw when used in furrows reduced infection of mung bean and cotton roots by soilborne root infecting fungi. Where seeds of soybean and wheat were sown after treatment with culture filtrates of microbial antagonists viz., A. candidus, G. virens, P. lilacinus and T. harzianum, infection of soybean and wheat roots by R. solani reduced by upto 34-80% and 0-42% respectively, whereas, M. phaseolina infection reduced by upto 35-100% both on soybean and wheat roots. These results suggest the involvement of toxic metabolites in disease supression. It indicates that for effective biological control, incorporation of antagonists previously multiplied on a suitable substrate may have better results. Our experiments on the delivery of antagonists also support this hypothesis where comparatively better results were obtained when microbial antagonists were used after multiplication on rice grains than their multiplication on wheat straw, rice straw, sorghum grains and coating seeds with microbial antagonists.

Soil amendment with proper organic material may affect root diseases by reducing number of fungal propagules through germination followed by lysis, temporarily or permanently inactivating fungal propagules in soil, or serving as a food base for antibiotics or toxin producers or in producing stimulatory or inhibitory volatile substances (Cook, 1977). It also influences soil physical characters which together with the nutrients released by decomposing organic matter results in a better plant vigour and in many diseases this can help the plants to resist attack of pathogen or replace the damaged roots quickly by new roots (Singh, 1983). Kannaiyan and Prasad (1981) found that Azadirachta indica and Ipomea crassicola reduced saprophytic ability of R. solani, while the population of R. solani is known to increase by clover straw and wheat straw (Moubasher & Abdel-Hafez, 1986) and corn tissue amendments (Lewis, 1976).

Addition of organic amendments are known to stimulate microbial activity in soil and supression of root rot of cotton was related to an increase in population of antagonistic bacteria and actinomycetes in amended soil (Ghaffar et al, 1969). Similarly, chlamydospore population of Fusarium spp., is known to reduce by conifer litter (Toussoun et al, 1969). In our experiments where lucerne, wheat straw and molasses were used @ 1 and 10% w/w for soil amendment, lucerne used @ 1 and 10% w/w respectively showed a reduction of 18 and 87% in M. phaseolina population after 15 days of amendment whereas after 30 days of amendment M. phaseolina population in 1 and 10% amendment was 14 and 24% greater than the initial populations. Combined use of lucerne (@10% w/w) and microbial antagonists viz., A. candidus C. cladosporioides and T. roseum respectively resulted in 94, 97 and 97% reduction in M. phaseolina population after 15 days but after 30 days population again showed an increase over the initial populations in all the treatments. It would suggest that a judicious and continuous incorporation of small amount of suitable organic substrate might result in a normal shift of equilibrium of soil microflora. The mechanism underlying this effect needs elucidation.

Sowing cotton mixed with moth (Vigna aconitifolia (Jacq.) Marechal) has been suggested to reduce incidence of cotton root rot (Vasudeva, 1941). The reason ascribed is that the canopy of moth foliage reduces the soil temperature. The foliage of the inter crop in generally removed after almost 8 weeks period leaving the stubbles in the field. This when incorporated in soil serves as organic substrate which brings about a shift in soil microflora resulting in reduction in M. phaseolina infection of cotton (Ghaffar et al, 1969). In the present study soil amended with moth @ 5% w/w showed a gradual decline in R. solani population where upto 36% reduction was observed after 12 weeks of amendmnts. This may be due to a general microbial balance of soil microorganisms but the use of moth @ 5% w/w would appear to be excessively high dose and may not be economical. Sowing mung bean in plots amended with moth @ 1% w/w reduced root infection by M. phaseolina and R. solani by 25 and 51% over the control after 2 weeks of amendment but after 4 weeks there was no significant difference in infection in amended and non-amended plots. It would suggest that periodic addition of small amount of organic substrate or integrated use of organic amendment with microbial antagonists would result in diseases control.

Artificial fertilization is a general practice to enhance plant growth and increase crop productivity since micro-organisms in soil are affected directly or indirectly by fertilization (Curl & Rodriguez-Kabana, 1973). In our studies, use of organic fertilizers viz., cow dung, sewage sludge and Zarkhez @ 1% w/w reduced R. solani infection on mung (50-67%) and cotton (36-50%) whereas no noticeable reduction was observed in M. phaseolina infection which may be attributed to high level of nitrogen which is known to increase susceptibility to M. phaseolina infection (Sivaprakasam et al, 1975; Erzhibov et al, 1980; Anabosur et al, 1977; Florya, 1974). Combined use of soil fertilization and seed treatment with microbial antagonists proved more effective than the use of organic fertilizers or microbial antagonists alone.

Resting propagules produced by soilborne root infecting fungi are capable of survival for long periods since in the present studies, use of fertilizers, antagonists and organic amendment at low dosages did not eliminate root infection by root infecting fungi. A combination of high soil moisture and organic amendment (low C:N ratio) did show promising results in the elimination of sclerotia of M. phaseolina from the soil (Sheikh & Ghaffar, 1980).

In recent past a new method of solar pasteurization of soil for disease control was developed by Katan et al (1976) in Israel. This technique was successfully used in our studies where population of M. phaseolina and R. solani declined to zero after 7 days (at 0-5 cm depth) of mulching treatment. Mung, sunflower and okra plants growing in mulched soil showed significantly less infection by R. solani, M. phaseolina and Fusarium spp. Loss in viability was related to high soil temperature which was attained after covering the soil with polyethylene sheet. In the present study maximum temperature of 48-52 °C at 5 cm depth is comparable with the daily occurrence of temperature of 49-52 °C in Israel. The soil from 15-20cm depth was brought back to the surface and sclerotial inoculum eleminated by subsequent mulching. Similar reduction in the population of Verticillium dahliae (Katan et al, 1976; Pullman et al, 1981), Fusarium oxysporum f.sp. lycopersici (Katan et al, 1976), Sclerotium rolfsii (Grinstein et al, 1979), Rhizoctonia solani, Thielaviopsis basicola and Pythium spp., (Pullman et al, 1981), Phytophthora cinnamomi (Pinkas et al, 1984), Sclerotium cepivorum, Sclerotinia minor (Porter & Merriman, 1983), Sclerotium oryzae (Usmani & Ghaffar, 1982) and M. phaseolina (Sheikh & Ghaffar, 1984) as well as the disease caused by these fungi have been reported.

The mechanism of solarization on fungus inhibition is not clearly understood, but it may involve enzyme inactivation, changes in fatty acids or membrane components (Crisan, 1973). Apart from direct thermal inactivation of fungal propagules, the possible role of biocontrol mechanisms can not be ruled out since a greater proportion of bacteria and actinomycetes inhibitory to M. phaseolina were found in heated soil than in natural soil.

Soil mulching treatment would appear superior to fumigation since it is safer to use. The effect of mulching of soil with selected antagonists with or without amendments and fungicides need further investigation. Time and application of mulching in wet soil during hot summer when it would be more efficacious can thus be predicted. A judicious application of mulching practice at proper time should give beneficial results.

There are many reports of increased disease severity by nematode-fungal association. Macrophomina phaseolina - Meloidogyne spp., combination is known to increase disease severity on okra and egg plant (Chhabra & Sharma, 1981), soybean (Agarwal & Goswani, 1973), jute (Majumder & Goswani, 1974) and french bean (Al-Hazmi, 1985), etc., whereas association of Rhizoctonia solani - Meloidogyne spp., was found more destructive on tomato (Chahal & Chhabra, 1985), potato (Sharma & Gill, 1979), okra (Golden & Van Gundy, 1985), french bean (Reddy et al, 1979) and many other crops. Similarly Minton et al (1975) reported increased susceptibility of soybean to Sclerotium rolfsii infection as a result of root knot infection, whereas several Fusarium spp., were found more severe in presence of root knot nematode on various plants (Menezes & Balmer, 1974; Ferraz & Lear, 1976; Sidhu & Webster, 1977). There are only few reports on the control of root rot and root knot disease complex. A combined use of nematicides and fungicides has been reported to decrease the severity of the disease (Abu Elamayem et al, 1978). Jatala (1985) reported the parasitism of Meloidogyne eggs by Paecilomyces lilacinus. In our experiments use of P. lilacinus for the control of root rot - root knot disease complex of mung, okra, sugarbeet and tomato showed promising results than use of fungicides viz., Agrosan and Vitavax and nematicides viz., Furadan and Mocap. Residual effect of P. lilacinus was also significant than Furadan used either alone or mixed with P. lilacinus. It was interesting to note that P. lilacinus was more effective against M. incognita than M. javanica. A judicious use of a pesticide and or

microbial antagonists could thus be used to eliminate root rot and root knot disease complex of crop plants.

Significant achievements and thrust for future

From an extensive collection of diseased specimens belonging to 91 different species of plants, several of the root infecting fungi were isolated and identified. This includes 52 new hosts of R. solani, 44 of M. phaseolina, 2 of S. rolfsii, 6 of B. theobromae, 44 of D. australiensis, 12 of D. halodes and 6 of D. state of C. spicifer not hitherto recorded. There is need that comprehensive study of soilborne root infecting fungi should be continued since fungi attacking above ground portions of plants have been reported and fungi inhabiting soil and affecting underground portions of plants have hitherto received little attention. With use of selective isolation techniques the presence of soilborne root infecting fungi like pythiacious fungi and species of Verticillium could be detected.

Whereas seperate use of the application of fungicides like Benomyl and Captan in soil were ineffective, their combined use reduced infection of roots by R. solani, M. phaseolina and Fusarium spp. A judicious application of fungicides could thus be developed. But since these fungicides are expensive or are known to produce environmental hazards, efforts should be made for integrating biological control agents in the control practices.

For the control of soilborne root infecting fungi we have isolated and used several biocontrol agents. Some of the biocontrol agents which have shown promising results in the control of soilborne root infecting fungi are A. candidus, G. virens, P. lilacinus, T. flavus, T. hamatum, T. harzianum, B. subtilis, Streptomyces sp., R. meliloti and R. leguminosarum. There is need to improve strains of antagonists or isolate new potential biocontrol agents from diseased tissues buried in soil. Addition of acidic fertilizers may change pH and nutrient status of soil so that growth of potential antagonists is favoured.

In our experiments some of the biocontrol agents have shown promising results in control of soilborne root infecting fungi. These have been used as seed treatment or applied in soil. Introduction of proper strain of antagonists at a time when the disease causing pathogen is more vulnerable might delay disease development.

The results of our experiments have showed that the effect of the application of biocontrol agent or the use of organic substrate into soil is short lived. The efficacy of biocontrol agent or the use of organic substrate could be enhanced by repeated application of biocontrol agent or repeated application of organic substrate into soil to enhance the activity of resident microflora resulting in biological control of soilborne root infecting fungi.

Of the various strains used in our experiments, we have found that P. lilacinus, T. harzianum, G. virens, A. candidus, B. subtilis, Rhizobium spp., and Streptomyces sp., were more effective in reducing soilborne root infecting fungi. Of these P. lilacinus is known to parasitize the eggs of Meloidogyne incognita the nematode causing root knot disease. We have found that P. lilacinus inhibited growth of root infecting fungi. P. lilacinus has also been effectively used to control root rot and root knot disease complex affecting crop plants. This is an important achievement not hitherto reported. Similarly, Rhizobium leguminosarum and R. meliloti, the bacteria which produce root nodules on leguminous plants and fix atmospheric nitrogen, inhibited growth of root infecting fungi in agar culture and in soil. There is thus great promise of the use of root nodule bacteria also in biological control of soilborne root infecting fungi.

We have found that microbial antagonists multiplied on rice grains were comparatively more effective than their multiplication on wheat straw, rice straw or sorghum grains, etc. There is need to develop techniques for the mass production of biocontrol agent and their delivery into soil. Economic feasibility on the production of microbial antagonists has to be worked out and the industrialists and commercial business concerns could become more interested to replace chemical pesticides which are so deleterious to the ecosystem.

Use of polyethylene mulching was found more effective in eliminating soilborne root infecting fungi. Whereas use of polyethylene mulching may not be feasiable over large area in the country, the mulching treatment can be effectively used atleast in nursery beds to eliminate transport and spread of initial inoculum into field.

We found that sewage sludge and other organic substrates like cow dung, buffalo dung, chicken manure, etc., significantly increased seed germination and reduced R. solani infection on okra, cotton, bottle gourd and mung. A judicious use of these fertilizers could be used in field

especially when combined use of organic fertilizers and microbial antagonists gave better results than their seperate use.

We have developed a diagnostic service for the identification and control of soilborne plant diseases. A 10 day training program on "Diagnosis and control of soilborne plant diseases" was organized in which research and extension workers and progressive farmers and representatives of pesticide firms participated. There is need to conduct such refresher courses for the research and extension workers at periodic intervals.

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ABSTRACTS OF PAPERS

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